RESEARCHES ON FUNGI

VOL. III.

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BY THE SAME AUTHOR

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RESEARCHES ON FUNGI

VOLUME III

THE PRODUCTION AND LIBERATION OF SPORES IN HYMENOMYCETES AND UREDINEAE

BY

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WITH TWO HUNDRED AND TWENTY-SEVEN FIGURES IN THE TEXT

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TO

WILLIAM B. GROVE

AUTHOR OF *THE BRITISH RUST FUNGI*

AS A MARK OF ESTEEM AND IN APPRECIATION OF HIS

ASSISTANCE IN PREPARING FOR THE PRESS THE

THREE VOLUMES OF THIS WORK

PREFACE

In the second volume of this work, published in 1922, the Agaricineae were divided into (1) the Aequi-hymeniiferae or Non-Coprinus Type of fruit-body organisation, made up of five Sub-types, and (2) the Inaequi-hymeniiferae or Coprinus Type, made of up six Sub-types ; and the Panaeolus Sub-type was described in detail. In the present volume, eight more of the Sub-types have been described, leaving two, the Plicatilis and the Curtus, for Volume IV.

The Coprini, or Ink Fungi, are among the most interesting of all Agaricineae, owing to the autodigestion of their gills, the dimorphism, trimorphism, or tetramorphism of their basidia, and the presence in many of them of very large cystidia. Every mycologist knows *Coprinus comatus*, *C. atramentarius*, and *C. micaceus*. I have attempted to show how the fruit-bodies of these and other Coprini, notably *C. sterquilinus*, carry out their one great function of producing and liberating spores.

Chapter XII treats of the bioluminescence of *Panus stypticus*, a fungus which is remarkable in having two physiological forms ; one, occurring in North America, which is luminous ; and the other, occurring in England and probably throughout Europe, which is non-luminous. The emission of light by decaying leaves is recorded for England, Canada, and the United States ; and shadow-photographs, made with the light of the leaves, are reproduced as illustrations.

Chapter XIII contains a review of our knowledge of the agarics which are parasitic on other agarics, and Chapter XIV an account of some observations, carried out in the open, in mid-winter, upon the nocturnal discharge of spores from the fruit-bodies of *Pleurotus ostreatus* and *Collybia velutipes*.

Part II is devoted to the Rust Fungi which, like the Hymenomycetes, have been considered from the point of view of the production and liberation of their spores. I have shown that the phenomena accompanying the development and discharge of basidiospores are exactly alike in the Uredineae and Hymenomycetes, thus strengthening the view that these two great groups are closely related. I have also described in detail, for the first time, my observations on the violent discharge of the basidiospores and aecidiospores of *Puccinia graminis*.

The volume contains two hundred and twenty-seven illustrations, including one hundred and fourteen drawings and one hundred and thirteen photographs. Fifteen of the drawings and fifteen of the photographs have been borrowed from other authors. The other ninety-nine drawings were executed by my own hand. For copying the drawings reproduced in Figs. 183, 188, and 191, my thanks are due to Dr. Nellie Carter. The source of each borrowed illustration is duly acknowledged in the text.

Of the ninety-eight photographs here published for the first time, sixty-six were made under my direction ; and the others were very kindly contributed by friends and correspondents : six by A. E. Peck ; four each by W. S. Odell and J. E. Titley ; three by Miss E. M. Wakefield ; two each by A. E. Field and W. F. Hanna, P. Grafton, J. P. Hillhouse, and Miss Irene Mounce ; and one each by Mrs. J. S. Bayliss Elliott, H. M. Fitzpatrick, H. T. Güssow, Somerville Hastings, the late W. Hillhouse, C. D. Learn, and H. R. Wakefield.

To numerous correspondents in both Europe and North America I am indebted for assistance rendered me by sending specimens, photographs of fungi, reprints of papers, and books, and by supplying information in response to various inquiries. In this connection my thanks are due especially to Miss E. M. Wakefield, M.A., of the Royal Herbarium, Kew, J. Ramsbottom, M.A., of the Natural History Museum, South Kensington, and Dr Howard A. Kelly of Baltimore. In the final revision of the proofs, W. B. Grove, M.A., to whom this volume is dedicated, has once more given me the benefit of his wide mycological knowledge and experience.

A. H. REGINALD BULLER.

WINNIPEG, *August* 19, 1924.

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PART I

THE PRODUCTION AND LIBERATION OF SPORES IN HYMENOMYCETES

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The Psathyrella Sub-type—*Lepiota cepaestipes*. General Description—The Spore-discharge Period—The Gills—The Hymenium—Correlation of Facts—The Cheilocystidia—The Scales on the Pileus—Irregularities in the Development of the Basidia—Remarks on *Lepiota procera*

The Psathyrella Sub-type.—The Psathyrella Sub-type of fruit-body possesses all the general characters already described for the Aequihymeniiferous Type : the gills are wedge-shaped in cross-section and positively geotropic, the hymenium looks more or less downwards to the earth, and every part of the hymenium (every square mm.) produces and liberates spores during the whole period of spore-discharge.

The Psathyrella Sub-type of organisation about to be described has been met with so far only in two species, namely, *Lepiota cepaestipes* and *Psathyrella disseminata*. Field observations show that the fruit-bodies of both these species open rapidly, shed all their spores in from twelve to twenty hours, and then die down. We may therefore conclude that the fruit-bodies of the Psathyrella Sub-type have a strictly ephemeral existence. It is very important to grasp this fact, for correlated with it are : (1) the light structure of the sporophores, (2) the special structure of the basidia and paraphyses, and (3) the mode of functioning of the hymenium as a whole.

The ephemeral nature of the fruit-bodies of the Psathyrella Sub-

type is exceptional for the Aequi-hymeniiferae. It is true that, in the Bolbitius Sub-type, the fruit-bodies are also ephemeral; but, in the Armillaria, the Inocybe, and the Panaeolus Sub-types which contain most of the Aequi-hymeniiferae, the fruit-bodies are persistent, *i.e.* they last longer than one day after commencing to shed spores, and their spore-discharge period varies from a few days to a week or even longer. As examples of these persistent species, which contrast with the ephemeral *Psathyrella disseminata* and *Lepiota cepaestipes*, may be mentioned: *Panaeolus campanulatus* which sheds spores in the laboratory for from 7 to 11 days, *Psalliota campestris* which sheds spores for from 5 to 6 days, *Marasmius oreades*, *Armillaria mellea*, Russulae and Lactarii.

Let us now consider in detail the special characters of the Psathyrella Sub-type. These are as follows:

(1) The whole structure of the fruit-body is relatively light and suited to a brief duration of the pileus.

(2) The spore-discharge period is very brief, its length varying from 12 to 24 hours.

(3) The gills are not mottled and there are no waves of development passing over the hymenial surface: the hymenium develops everywhere in a very even manner. In this respect we have a distinct contrast with the Panaeolus Sub-type.

(4) The number of generations of basidia on the hymenium is limited to three or four. In the Panaeolus Sub-type there are at least eight such generations and probably ten or twelve.

(5) On any small area of the hymenium, the basidia of one generation do not wait to develop spores until the basidia of the previous generation have discharged their spores: there is overlapping in development. The second-generation basidia begin to develop spores some hours before the spores of the first-generation basidia have ripened and have begun to be discharged. The third-generation basidia behave to the second-generation basidia, and the fourth to the third, as the second to the first. The spores of the fourth-generation basidia may be in course of development before the spores of the first-generation basidia have all been discharged. This overlapping in the development of the successive generations of basidia does not occur in the Panaeolus Sub-type.

(6) The basidia are polymorphic, for they consist of four distinct types, each type being distinguished from the other types by a difference in length. The longest basidia belong to the first generation, the next longest to the second generation, the next longest to the third generation, and the shortest to the fourth generation. With difference in length of the basidia is associated a difference in protuberancy of the basidium-bodies above the general surface of the hymenium as defined by the outer walls of the paraphyses. The first-generation basidia (the longest set) protrude farthest, the second-generation basidia (the next longest set) protrude not quite so far, the third-generation basidia (the next longest set) protrude still less far, and the fourth-generation basidia (the shortest set) do not protrude at all.

The grouping of the basidia into four sets, each set having a different degree of protuberancy beyond the general level of the hymenium, is a unique feature, so far as the Aequi-hymeniiferae are concerned, and one which serves to separate at once the Psathyrella Sub-type from the four other Sub-types with which it is associated. However, in the Inaequi-hymeniiferae (Coprini), the basidia are usually dimorphic, and sometimes trimorphic or tetramorphic.

(7) The basidia of the various generations are so crowded together that it often happens that the spores of the older and more protuberant basidia more or less overstand the spores of the younger and less protuberant basidia without touching them. This crowding of the basidia is only rendered possible without mishap to the spores by the differential protuberancy of the bodies of the basidia of successive generations. It permits of an increase in the number of spores produced on any unit area of the hymenium and must therefore be regarded as an important factor in fruit-body efficiency. A similar crowding of the basidia is characteristic of all Inaequi-hymeniiferae, *i.e.* of all the Sub-types of the Coprinus Type.

(8) The paraphyses are relatively large and well-developed. They are joined together laterally into a system of cells, which constitutes a pavement through which the isolated basidia protrude. Since successive generations of basidia develop their spores at the same time, the paraphyses (sterile elements) are necessary as space-making constituents of the hymenium. As space-makers

they separate adjacent basidia and thereby prevent the mutual jostling of the spores. We have similar large paraphyses forming a hymenial groundwork in the Bolbitius Sub-type and in the Inaequihymeniiferae where the fruit-bodies are also very ephemeral; but in the Panaeolus, the Armillaria, and the Inocybe Sub-types the paraphyses are more or less isolated from one another and do not form a continuous and well-marked system.

The name *Psathyrella* Sub-type has been chosen in preference to *Lepiota cepaestipes* Sub-type, firstly, because *Psathyrella disseminata*, on which it is partly based, is so widely distributed and well-known, and, secondly, because the fruit-body mechanism of *Lepiota cepaestipes* is not characteristic for all the species of Lepiota.

Concerning the well-known fruit-bodies of *Lepiota procera* something will be said at the end of this Chapter. It will therefore be sufficient here to state that the organisation of the hymenium of *L. procera* is entirely different from that of *L. cepaestipes* and resembles rather that of the Panaeolus Sub-type than that of the Psathyrella Sub-type.

Lepiota cepaestipes and *Psathyrella disseminata* are not necessarily closely related, notwithstanding that the arrangements for procuring the production and liberation of their spores are almost identical. Indeed, I am of opinion that the resemblance in their fruit-body mechanism is due rather to evolutionary convergence than to direct descent.

The fruit-bodies of *Lepiota cepaestipes* and *Psathyrella disseminata*, which illustrate the Psathyrella Sub-type, will be described in detail in this Chapter and the next. Since the available illustrations happen to be somewhat more numerous for the Lepiota than for the Psathyrella, the first-named species will be described first.

Lepiota cepaestipes. General Description.—The fruit-bodies upon which my observations were made were found in the forcing chamber of a hot-house at Birmingham, England. They were coming up in small groups on the top of a thin layer of cinders, which contained a certain amount of peat and Sphagnum-moss. Below the cinders was some rotting wood which in all probability provided

the nutrient substratum for the mycelium. The fungus appeared in the same situation during several successive years. The photographs in Figs. 1 and 2 show the external form and size of some

FIG. 1.—*Lepiota cepaestipes*. Two fruit-bodies coming up on cinders, etc., from a hot-house in England, photographed about noon. The pilei rapidly expanded in the evening, shed spores during the following night and the forenoon of the next day, and finally collapsed during the succeeding afternoon. Natural size.

typical fruit-bodies, whilst the drawings in Fig. 3 show some corresponding median vertical sections.

The fruit-bodies of *Lepiota cepaestipes* are gregarious and, in England, appear on cinders, peat, etc., covering rotting wood in hot-houses, but never in the open. The species has probably been introduced with greenhouse plants from abroad. In Ceylon it occurs in the open, and there the fruit-bodies are always yellow and

never white,[1] in this respect resembling the fruit-bodies which I found at Birmingham.

A very young fruit-body is shaped like an hour-glass and the diameter of the swollen stipe equals that of the unexpanded pileus.[2]

The pileus, when very young, is ovate or barrel-shaped and nearly twice as long as broad. Before expansion, it may attain a height of 1 to 1·25 inches. On expanding, it becomes campanulate and then obtusely conical, but never plane. The disc remains prominent and the general form of the pileus much resembles that of *Lepiota procera*. The width of the expanded pileus varies from about 2 to 3 inches. The pileus-flesh is thin except at the disc where it may attain a thickness of close upon 0·1 inch. It almost disappears toward the pileus-margin which, after expansion, becomes semi-transparent and plicate. The surface of the pileus is mealy and squamose with scattered plumose scales, especially at the disc: and its general colour is pale lemon-yellow, the scales being somewhat darker or more sulphur-yellow. The scales can be easily removed from the pileus as coarse dust by rubbing the surface with a finger.

The gills are at first quite vertical and appressed to the stipe, as in the genus Coprinus (Fig. 3). At their inner extremities next to the top of the stipe, they soon become free; and, as the pileus expands, they become separated from the stipe by a circular space. They are pale yellow, ventricose, about 1 inch long, and from 0·2 to 0·3 inch wide in the middle. They are also extremely thin but, at the same time, tough and flexible: they can be bent by the fingers without tearing.

The stipe is about 3 inches long, straight or curved upwards, firm, almost smooth but slightly pruinose; narrow, 0·1 to 0·2 inch wide, and pale yellow at the top; but decidedly ventricose, 0·4 to 0·5 inch wide, and more sulphur-yellow below; hollow above but solid near the base. The annulus is perfect, erect, persistent, and distant from the base of the stipe.

The spores are colourless, smooth, oval, 7–8 μ long, 5 μ wide, and,

[1] T. Petch, "European Fungi in the Tropics," *Trans. Brit. Mycological Soc.*, vol. iii, 1911, p. 342.

[2] Petch makes a similar remark for his Ceylon specimens. *Ibid.*

when allowed to accumulate on a dark surface, give rise to a white spore-deposit.

The whole fruit-body has a peculiar and rather unpleasant odour. On collapsing, the pileus, especially at the disc, turns more or less

FIG. 2.—*Lepiota cepaestipes*. Two fruit-bodies from a hot-house in England, photographed at 11.30 A.M. The younger fruit-body opened during the succeeding evening. The older one, which is fully expanded, opened on the previous evening, shed its spores in the night and early morning and is about to collapse. Natural size.

brownish in colour. The expansion of the pileus usually begins in the afternoon, is completed in the night, and the collapse of the fruit-body takes place the following afternoon or evening. The spores are chiefly shed at night and in the early morning.

The white form of the fruit-bodies of *Lepiota cepaestipes*, which is illustrated in Cooke's *Illustrations of British Fungi* (Plates 5 and

942), I have never seen. My specimens resemble more or less Cooke's left top figure in his Plate 5. His fruit-bodies do not appear to be fully expanded ; but, for young fruit-bodies, they are too brown at the disc. According to my experience, the discs of young fruit-bodies are deeper yellow than the rest of the pileus, owing to the sulphur-yellow of the scales there ; and they become brown only when the fruit-body is dying.

According to Petch, who has found *Lepiota cepaestipes* growing in the open in Ceylon, the middle and lower figures in Cooke's *Illustrations*, Plate 1179, are ascribed by Cooke to *Lepiota licmophora* erroneously, and really represent *L. cepaestipes*. The other figures in the same Plate, according to the same critic, are badly copied drawings of *L. licmophora* with the original colours altered.[1] The middle and lower figures of Plate 1179 resemble my specimens of *Lepiota cepaestipes* in both colour and general form, although their stipes are longer and less swollen below.

The Spore-discharge Period.—In order to make some precise observations on the length of the spore-discharge period of *Lepiota cepaestipes*, I proceeded in the following way. One afternoon, three fruit-bodies were coming up in the greenhouse and, toward evening, their pilei were beginning to expand. At 6.30 P.M. one of the fruit-bodies, together with a little of the substratum containing the mycelium, was dug up and then planted very carefully in its original upright position in a cup containing wet peat ; whereupon the cup was taken into a house and set in a saucer containing water. Next, for the purpose of catching any falling spores, a glass slide was placed on a support resting on the cup, so that there was a clean glass surface just beneath the gills (but not touching them) on one side of the pileus. The whole was then covered with a large bell-jar.

At 7.15 P.M. the slide was removed and examined with the microscope. No spores had settled upon it : this indicated that the spore-discharge period had not yet begun. The slide was put back under the pileus. At 7.50 P.M. it was re-examined and a few spores were found upon it. We may suppose, therefore, that spore-discharge had begun about 7.45 P.M. A second slide was placed

[1] T. Petch, *loc. cit.*, p. 346.

beneath the pileus. At 4 A.M. a very thick deposit of spores was found to have accumulated upon it during the night. A third slide was placed beneath the pileus. At 8 A.M. another thick spore-deposit had been formed, but not so thick as its predecessor. A fourth slide was placed beneath the pileus. At 9.30 A.M., after an hour and a half, the new spore-deposit was so thin that it outlined the gills but very faintly; it therefore seemed evident that the

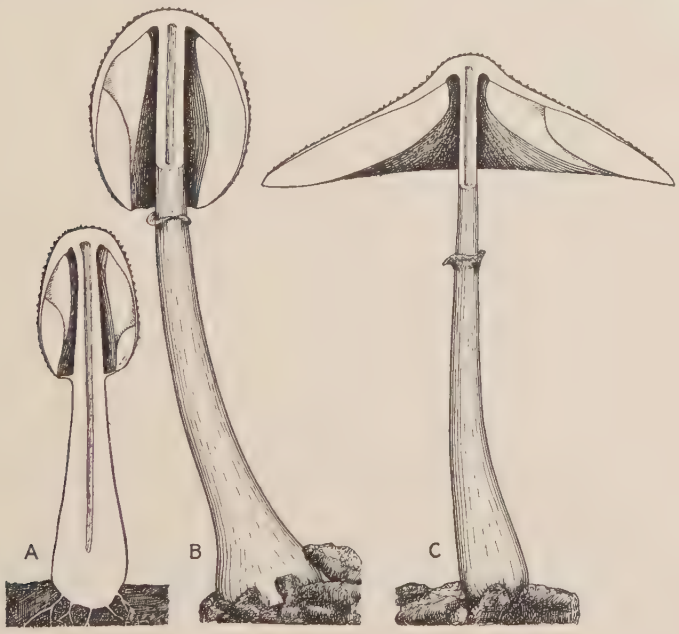


FIG. 3.—*Lepiota cepaestipes.* Sections of three fruit-bodies coming up among cinders, Sphagnum, etc., in a hot-house. A, in the morning, the gill-chamber still intact. B, in the afternoon, the pileus beginning to expand, an annulus left upon the stipe. C, at night, the pileus fully expanded and shedding spores. Natural size.

discharge of the spores was rapidly ceasing. A fifth slide was set beneath the pileus, and examined at 10.30 A.M. On the upper surface the fallen spores were so few that they could be counted easily. On a sixth slide, between 10.30 A.M. and 11 A.M., only a dozen spores fell.

The above observations show that the spore-discharge period for the fruit-body investigated began about 7.45 in the evening and

ended about 10.45 the next morning, and was therefore only about 15 hours long.

The two fruit-bodies left undisturbed in the greenhouse were tested for spore-fall in the morning. Between 10 and 10.30 A.M., a thin but distinct deposit of spores accumulated on slides placed beneath the pilei. Between 10.30 and 11 A.M., a very faint spore-deposit was formed. Between 11 A.M. and 12 noon, a few scattered spores fell. Assuming that the beginning of the spore-discharge period for the undisturbed fruit-bodies took place at the same moment as for the fruit-body removed to the house. we may conclude that the length of the spore-discharge period for the undisturbed fruit-bodies was slightly over 15 hours.

By 3 P.M., *i.e.* about 4 hours after the cessation of the discharge of spores, the fruit-bodies in the greenhouse were observed to be withering : their pilei were sinking downwards. At 7 P.M., one fruit-body had already collapsed, its stipe having broken across in the middle, and the other seemed to be tottering as if about to fall. Next morning both were completely collapsed and appeared to be dead.

These observations demonstrate how brief is the duration of the period of spore-discharge in the fruit-bodies of *Lepiota cepaestipes*, and for how short a time the expanded pilei are spread out before collapsing. We shall see that with this ephemeral existence of the fruit-bodies the general structure and the mode of functioning of the gills is strictly correlated.

The Gills.—While the pileus is still barrel-shaped and as yet unexpanded, the gills are closely packed together. The packing is shown in the transverse section through a young pileus illustrated in Fig. 4. As the pileus opens, the gills become drawn apart and, when the spores are being shed, the interlamellar spaces provide ample room for the successful escape of the spores (Fig. 5). Each gill is wedge-shaped in cross-section (Figs. 4 and 5) and positively geotropic so that, as shown in Fig. 5, every part of the hymenium in a normally oriented fruit-body comes to look more or less downwards. The spores are discharged with violence from the basidia, as in other Hymenomycetes. Their trajectories have been represented diagrammatically in Fig. 5. The horizontal part

of each sporabola has been supposed to be about 0·1 mm., as in the Mushroom. From the fact that the spores measure 7–8 by 5 μ and from the observations of the rates of fall of spores of various sizes given in Volume I.,[1] it may be inferred that, in still air, the rate of fall of the spores of *Lepiota cepaestipes* in a vertical direction between the gills is about 1 mm. per second. At this rate of fall, a spore would take 4 seconds to fall from the top to the bottom of one of the interlamellar spaces shown in Fig. 5.

Fig. 4.—*Lepiota cepaestipes.* Part of a transverse section through an unexpanded pileus (*cf.* Fig. 2, left), showing: packing and shape of the gills, and the nature of the trama and pileus-flesh. The sterigmata were just beginning to develop on the most protuberant basidia. Magnification, 15.

The pileus-flesh is composed of large, loosely-compacted hyphae between which are well-developed interhypal air-spaces (Fig. 5). Similar hyphae are to be found in the trama of the gills, but here they are placed in more or less transverse positions, so that they cross the mid-plane of each gill from one subhymenium to the other. In *Lepiota procera* the tramal elements have an approximately similar disposition (Fig. 17, B, p. 27). By reference to Fig. 9 (p. 16), it will be seen that the trama has the lightest possible construction: its organisation reminds one of the trellis-work or web in the girders of iron bridges; although, of course, it is not in the least mathematically regular. Each gill can be divided into hymenium, subhymenium, and trama.

Very thin transverse sections through parts of the gills, made when the pileus is beginning to expand, take on shapes like those shown in Fig. 6. A study of such sections teaches us that, in the uninjured gills, there are certain strains and stresses normally set up, which make for gill-rigidity and which are of importance in the expansion of the pileus. Strips of the hymenium and subhymenium,

[1] These *Researches*, vol. i, 1909, p. 175.

when isolated from the trama, curl up in the manner shown at D. Some of the strips so curled up resemble watch springs. The hymenium, when given the opportunity, expands more than the underlying subhymenium. When forming part of a normal gill, therefore, it must be in a state of compression. Where a transverse section does not extend to the gill-edge, as at A, or is taken out of a gill, as at C, the opposing outer strips (made of hymenium and subhymenium), where not attached to tramal cells, curl over toward each other and become crossed. It is evident that the two opposite outer sheets of each gill tend to become convex toward the inter-lamellar spaces which they face. In an intact fruit-body, however, they are prevented from attaining this convexity because: (1) at their bases, along the line of the free gill-margin, they are joined to each other in pairs, (2) they are held in check in their middle parts by the transverse tramal cells, and (3) at their tops, where they adjoin the pileus-flesh, they are continuous with similar sheets on adjacent gills. The sheet of cells, which makes up the hymenium and subhymenium on the under side of the whole pileus, is corrugated symmetrically about the pileus-centre. The stresses and strains in the sheet during the expansion of the pileus are such as tend to flatten out the corrugations and, doubtless, to some extent at least, they assist in the expansion of the pileus and the separation of the gills from one another. The tendency which the sheets have to create interlamellar spaces may be gathered from the loop which has been formed between the two gills shown in

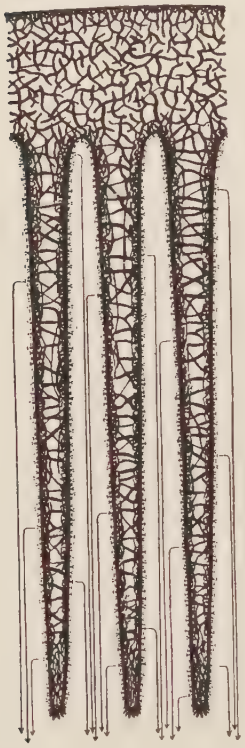


FIG. 5.—*Lepiota cepaestipes*. A vertical section through three gills of an expanded pileus (*cf.* Fig. 2, right), during the discharge of the spores. The gills are wedge-shaped and look vertically downwards. There are marginal hairs (cystidia) at the free margins of the gills. The trajectories of a number of spores which have been shot from the hymenium are indicated by the arrows. The peculiar structure of the trama and the loose texture of the pileus-flesh are indicated. Magnification, 20.

Fig. 6, B. Although the gills are very lightly built and very thin, they are fairly rigid. The rigidity, in part at least, must be due to the play of the opposing strains and stresses which have been discussed. In this respect we have an analogy with the rigidity which is found in young green shoots of Flowering Plants.

The subhymenium is composed of relatively small cells of various shapes. To the innermost ones are attached the large tramal cells, and from the outer ones spring the elements which make up the hymenium.

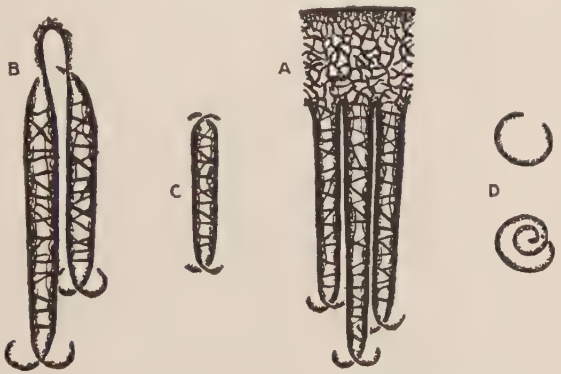


Fig. 6.—*Lepiota cepaestipes.* Thin sections taken transversely through an unexpanded pileus, lying in water. Effects of tensions in the gills. A, pileus-flesh with three gills attached. The two sides of each gill below have curled in opposite directions. B, two gills showing the same curling as in A; where the gills are connected, a loop has been formed which tends to push the gills apart. C, fragment of a gill in which the curling of the sides is seen at both ends. D, strips of hymenium, together with the subhymenium, which have curled up, the lower one into a spiral. Magnification, 15.

The Hymenium.—The hymenium on the sides of the gills is composed of two perfectly definite and easily distinguishable kinds of cells — the basidia and the paraphyses. Cystidia are absent from the gill-sides but form a fringe along the free gill-margins. To these fringing cystidia we shall return later on.

The general surface of the hymenium, after the spores have all been shed, presents the appearance shown in Fig. 7 at B. It is evident that the groundwork of the hymenium is made up of the paraphyses. These form a firm pavement in which, at fairly regular intervals, are set the projecting basidia. As if to strengthen the hymenium, adjacent paraphyses are welded together, and they therefore all form part of a continuous membrane. Each basidium is surrounded and isolated by four or five paraphyses, so that no two basidia ever come in contact with each other. Small areas

of the hymenium frequently exhibit a fairly regular quadratic arrangement of the elements. This is well shown in the *camera-lucida* drawing reproduced in Fig. 8. Here, the paraphyses on

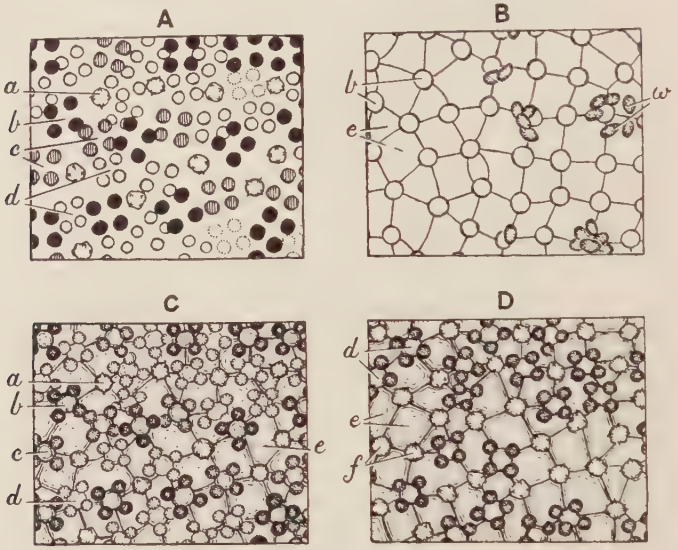


FIG. 7.—*Lepiota cepaestipes.* Surface sections of the hymenium. A, *camera-lucida* drawing showing: first-generation basidia, *a*, which have shed their spores, each with four sterigmata; the spores of the second-generation basidia, *b*, (shown black); the spores of the third-generation basidia, *c*, (shaded with lines); and the spores of the fourth-generation basidia, *d*, (unshaded). B, *camera-lucida* drawing of a piece of hymenium showing position of the elements after the cessation of spore-discharge. The hymenium is composed of a pavement of paraphyses, *e*, in which are set the collapsed basidia, *b*. Adhering to it are a number of wasted spores, *w*. C, the same as A, with the basidia and paraphyses added semi-diagrammatically. D, the same as C with spores left on the basidia of the fourth generation only; *d*, fourth-generation basidia; *e*, paraphyses; *f*, collapsed basidia of the first three generations. Magnification, 350.

several areas are square in outline and tend to form a pattern like that on a chess-board. The basidia are arranged in lines, one being set wherever four paraphyses meet. Where two chess-board areas meet, the junction is effected by elements which are arranged less regularly. Often the junction-paraphyses have three or five sides instead of the usual four. From studies such as that illustrated in Fig. 8, one gains the impression that the chess-board-like areas

develop independently of each other so far as the arrangement of the elements is concerned. The manner in which any single chess-board arrangement is brought into existence requires further investigation.

The basidia are produced in a series of four successive generations (Fig. 9). For one and the same generation, the basidia are about equally protuberant: but for the different generations, the average degree of protuberancy is strikingly different. The first-generation basidia are the longest and project farthest above the

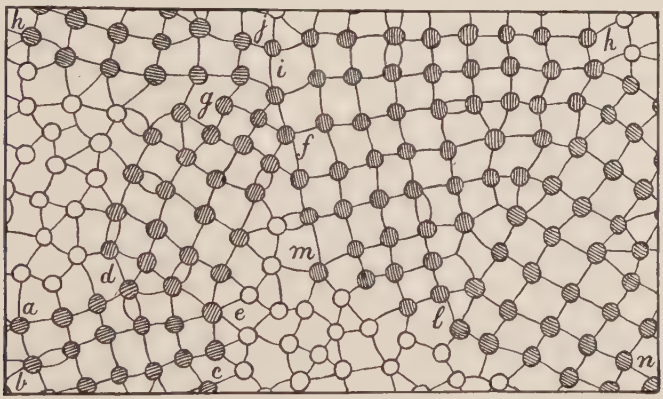


FIG. 8.—*Lepiota cepaestipes.* Surface view of the hymenium equal to about one-third of a square mm., drawn with the *camera lucida*, to show positions of paraphyses and basidia. There is a tendency for the mosaic-work to be arranged rectilinearly on certain areas, as is indicated by the shading. Such areas are: *a b c d, d e f g, h i j, i j k l m f, k l n.* There are irregularities in the shapes of the paraphyses where the areas join. Magnification, 350.

general surface of the hymenium. The fourth-generation basidia are the shortest and scarcely project at all. The second-generation and third-generation basidia form steps between the first and fourth. The first-generation and second-generation basidia differ least in protuberancy.

A stage in the development of the hymenium is shown in Fig. 10. Here it will be seen that the longest basidia, *b b*, have full-grown spores, that the intermediate basidia, *c c*, are still developing their spores, and that the shortest basidia, *d d*, have not yet developed even sterigmata. It is evident that, while the different generations of basidia are developing at the same time, the mid-basidia are a

stage behind the long basidia, and the short basidia a stage behind the mid-basidia. The basidia drawn in Fig. 10 represent the second, third, and fourth generations only. Basidia belonging to the first

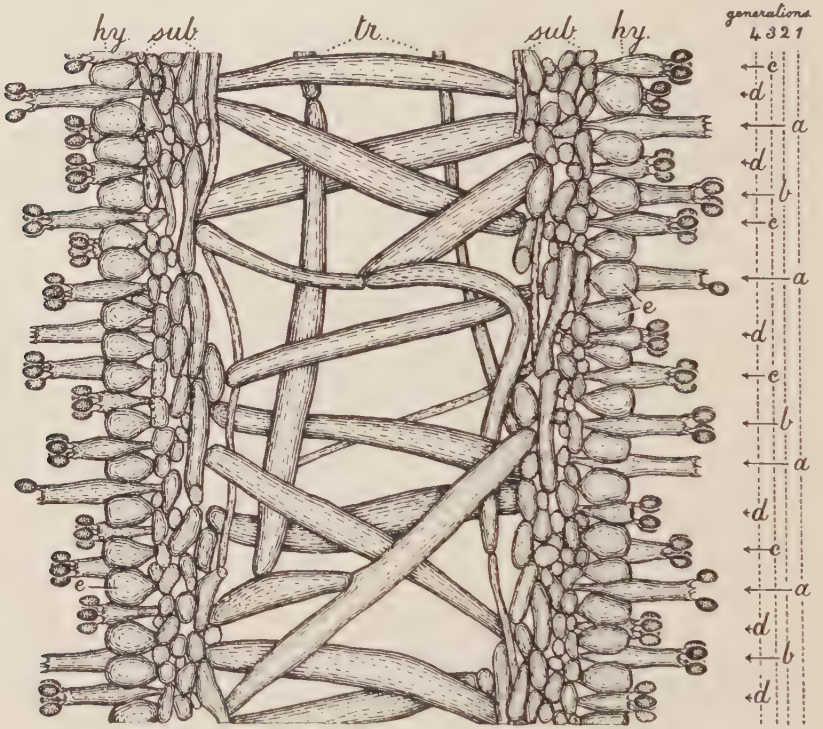


FIG. 9.—*Lepiota cepaestipes.* Cross-section of part of a gill showing : the hymenium, *hy* ; the subhymenium, *sub* ; and trama, *tr*. The trama has a peculiarly loose texture and is chiefly composed of large transverse elements. The hymenium is composed of paraphyses, *e*, and of basidia. The basidia are quadrimorphic : they exhibit differential protuberancy. Those of the first generation, *a*, are discharging their spores. The spores of the second-generation basidia, *b*, will be discharged next, then those of the third-generation basidia, *c*, and finally those of the fourth-generation basidia, *d*, Magnification, 350.

generation were not seen in the section from which the drawing was made.

The distribution of the basidia of the four generations in the hymenium is shown in the *camera-lucida* drawing reproduced in Fig. 7 at A (p. 14). The first-generation basidia have just shed their spores and are collapsing : they are represented with their now vacant sterigmata. The basidia of the other generations are repre-

sented only by their spores. The spores of the second-generation basidia are all sketched in uniform black. The spores of the third-generation basidia are all shaded, while those of the fourth-generation basidia are left as plain circles. At C in Fig. 7 are shown the same spores and first-generation basidia as are present at A ; but, in addition, the bodies of all the basidia of the second, third, and fourth generations, together with a background of paraphyses, have been diagrammatically added in order to complete the figure. At D is shown the same figure as at C, but in this only the fourth-generation basidia are represented as bearing spores : it is here to be supposed that the first-, second-, and third-generation basidia

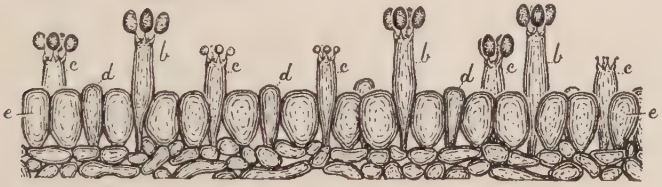


FIG. 10.—*Lepiota cepaestipes.* A stage in the development of the hymenium. No basidia of the first generation were observed. Basidia of the second generation, *b*, bear full-grown spores ; those of the third generation, *c*, are developing spores ; whilst those of the fourth generation, *d*, have not yet developed sterigmata. *e*, paraphyses. Magnification, 350.

have already shed their spores. Actual areas of the hymenium, like that shown at D, were observed upon fruit-bodies toward the end of the spore-discharge period.

Two important facts are brought out by such studies of the hymenium as those just described. The first is that the spores are discharged by the different generations of basidia according to their age. This can be put another way : the longest basidia shed their spores first, then the next longest, then the next longest and, finally, the shortest basidia or those of the fourth and last generation. The second fact of importance is that, notwithstanding the relatively large size of the paraphyses, the basidia of the four generations are so crowded on any given area of the hymenium that, *if they were all of exactly the same length, the spores of adjacent basidia would frequently touch and jostle one another*, so that there would be serious interference with their development and discharge. The *camera-*

lucida drawing in Fig. 7 at A (p. 14) shows that the spores of basidia belonging to an older generation frequently stand in part vertically above the spores of a younger generation.

Correlation of Facts.—We are now in a position to correlate all the different facts connected with the organisation of the hymenium. The fruit-body of *Lepiota cepaestipes* is adapted to shed its spores in little more than 12 hours, and with this brief spore-discharge period is correlated its ephemeral existence. On account of its being ephemeral, it does not need to be as rigid as equally large fruit-bodies belonging to other species which must persist for many days before all their spores are shed. Hence the pileus is provided with little flesh, and this has a very loose texture. For the same reason, the trama of the gills is also extremely light in construction. The fruit-bodies expand their pilei in the evening and shed their spores at night and in the early morning. At night there is no hot sun to be encountered and the air is much more saturated with moisture than in the day, so that the fruit-bodies open when there is least danger of too rapid transpiration. This periodicity of development, which corresponds with that of several small and delicate species of Coprinus, *e.g. C. ephemerus*, seems, therefore, to have a biological significance : it protects the lightly constructed fruit-bodies from transpiration dangers. Now, granted that the spores are to be discharged in a little over twelve hours, it is fitting that the development of the different generations of basidia should take place in a manner differing from that with which we became acquainted in our studies of the Panaeolus Sub-type. Instead of there being a large number of generations (eight to twelve), the generations have been reduced to four only. Not only this, but the development of successive generations has been hastened. Instead of a succeeding generation of basidia waiting until the discharge of the spores of the preceding generation has been accomplished before producing its own spores, as happens in fungi of the Panaeolus Sub-type, we find in our Lepiota that, shortly after the first-generation basidia have begun to develop spores, the second-generation basidia begin to develop spores also. Indeed, all the four generations of basidia in our Lepiota may be developing and maturing spores at one and the same time, although the first-generation

basidia always keep slightly in advance of the second, the second slightly in advance of the third, and the third slightly in advance of the fourth. The general result is that the four generations of basidia produce their spores in a much shorter space of time than would be taken if the method of development which we find in fruit-bodies of the Panaeolus Sub-type were adopted. The spores of the four generations reach maturity and are discharged in rapid succession in correspondence with the times when they begin their development. The following of one generation close behind another in the maturing of their spores makes the brevity of the spore-discharge period possible, and is evidently related to the ephemeral existence of the expanded pileus. The production of four generations of basidia with differential protuberancy enables more spores to be safely produced on, and discharged from, any given area of the hymenium during the short spore-discharge period than would be possible were the basidia all of equal length ; for, were they all of equal length, they would require to be less crowded or there would be jostling of the spores borne on adjacent basidia. The differential basidial protuberancy is therefore an economical arrangement : with it many more spores can be produced by the fruit-body than would be possible without it. We have thus been able to connect the polymorphism of the basidia with the general requirements of the fruit-body as an ephemeral organ. One other fact requires to be explained, namely, the large size of the paraphyses as compared with those of the Panaeolus Sub-type. The paraphyses in *Lepiota cepaestipes* have two functions : (1) they help to support the basidia in a vertical position, so that the spores are freely developed and subsequently discharged into the interlamellar spaces, and (2) they act as space-making agents, *i.e.* they separate the basidia and keep them at such distances apart that the spores of adjacent basidia develop without any mutual interference. If one were to take away the paraphyses in Fig. 7, C (p. 14), and set the basidia in contact, there would result such a crowding of the basidia that few spores could be properly developed and discharged. Even if the diameters of the paraphyses were reduced to one-half, the beautiful freedom of each separate basidium would be seriously hampered. It is evident that the paraphyses are structures which

make an important contribution to the efficient organisation of the hymenium.

The Cheilocystidia.— Along the free margin of each gill (Fig. 11, *a* and *b*) there is a fringe of irregularly clavate sterile cells which, using the terminology of Topin[1] and Knoll,[2] might be called *cystidiform cells*. However, for reasons which will be more fully explained in connection with a description of *Psathyrella dissemi-*

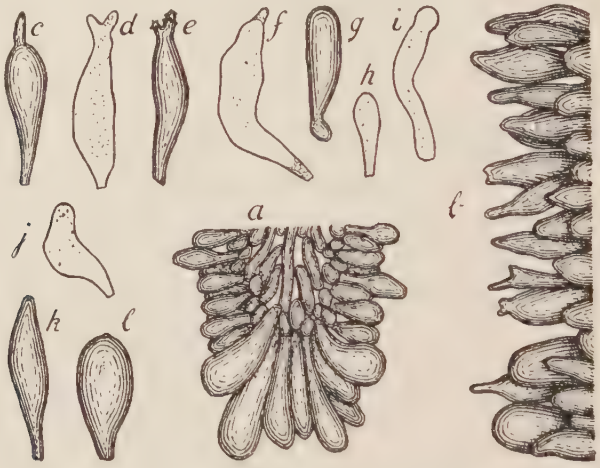


Fig. 11.—*Lepiota cepaestipes*. Cystidia on the gill-edge. *a*, transverse section through the edge of a young gill with the hymenium developing. The larger cells below are cystidia. *b*, a gill-edge seen from one side of the gill and showing the projecting cystidia. *c*—*l*, isolated cystidia of different forms; *c* and *f* are unicornuate; *d* and *e* bicornuate; *d*, *f*, *h*, *i* and *j* show contents composed of particles which exhibited Brownian movement. Magnification, 350.

nata, I prefer to call them *cheilocystidia*, *i.e.* cystidia produced on the free edge of a gill. The cheilocystidia of *Lepiota cepaestipes* have free ends which may be rounded, pointed, or extended into one or two short horns. The horns may be either smooth or coated with small particles (Fig. 11, *c* to *l*). The cheilocystidia are by no means of uniform size, for their length varies from 40 to 70 μ and their width from 10 to 20 μ. Each contains a large vacuole in which is

[1] J. Topin, *Notes sur les Cristaux et Concrétions des Hyménomycètes et sur le Rôle Physiologique des Cystides*, Thèse, St. Germain-en-Laye, 1901, p. 29.

[2] F. Knoll, "Untersuchungen über den Bau und die Function der Cystiden und verwandter Organe," *Jahrb. f. wiss. Bot.*, Bd. 50, 1912, p. 453.

situated a number of yellow-brown particles, possibly oil drops, which are in the most lively Brownian movement (Fig. 11, *d*, *f*, *h*, *i*, *j*). The function of the cheilocystidia is not apparent and not easy to explain. No direct evidence is forthcoming in support of the view that they are hydathodes. It must be remembered, however, that the fringe of cheilocystidia occurs on those parts of the gills which are developed in close proximity to the stipe, and which are most exposed to transpiration when the pileus expands. Possibly, for one or both of these reasons, the gill-edges, here as in

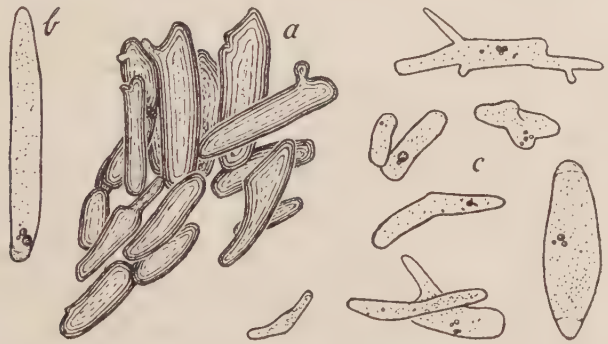


FIG. 12.—*Lepiota cepaestipes.* Cells making up the loose scales on the surface of the pileus (*cf.* Fig. 2). *a*, a group of cells showing exterior form; *b* and *c*, individual cells showing contents. The smaller particles in *b* and *c* were yellow and exhibited lively Brownian movement. The larger were yellowish-brown and exhibited slower Brownian movement. Magnification, 350.

the Coprini and the Panaeoli, etc., are left unprovided with basidia. The cheilocystidia may therefore be regarded tentatively as specialised hymenial cells simply filling up a space which for one or more reasons cannot be used satisfactorily for the production of spores. If the cheilocystidia are merely space-fillers or packing cells, their particular shape is not of much importance, and this may perhaps account for the great variability of the cheilocystidia of *Lepiota cepaestipes*.

The Scales on the Pileus.—The pileus, as we have seen, is covered with delicate plumose scales. Each scale is made up of elongated cells which easily separate from one another in water (Fig. 12). The cells vary from about 30 to 90 μ in length and from 5 to 25 μ in

width. Many of them are about 40 μ long and 15 μ wide. Just as for the cheilocystidia, the scale-cells contain each a large vacuole suspended in which are pigmented particles exhibiting the liveliest Brownian movement (Fig. 12, *b* and *c*). The particles are of two classes : numerous very small yellow ones and a few larger yellow or yellow-brown ones. The former show very active Brownian movement and the latter, as might be expected from their larger size, a slower movement. At the ends of some of the cells there is a small mass of motionless protoplasm. By comparing the descriptions and illustrations of the scale-cells and the cheilocystidia, it will be seen that there are similarities in size and contents for these two kinds of elements, which mark them out as being moulded by common developmental forces.

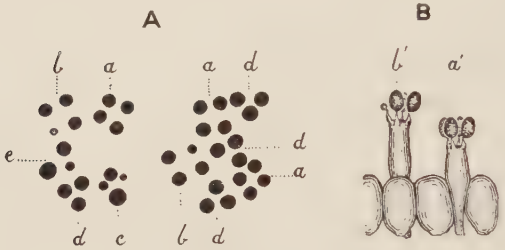


FIG. 13.—*Lepiota cepaestipes.* Abnormalities in spore-production. A, a surface view of hymenium showing spores of different sizes ; *a*, four normal spores of one basidium ; *b*, three normal spores and one aborted ; *c*, one spore normal, one enlarged, two aborted ; *d*, three spores of equal size ; *e*, three spores all differing in size. B, a cross-section of a hymenium with a basidium, *a'*, bearing four normal spores, and a basidium, *b'*, bearing three normal spores and one aborted. Magnification, 350.

Irregularities in the Development of the Basidia.—Certain irregularities made themselves apparent in a fruit-body which had been gathered in a hot-house and which developed its spores whilst its stipe was placed in water in a room. Most of the basidia produced four spores in the usual manner (Fig. 13, A, *a* ; B, *a'*). A few basidia, however, produced three equal-sized spores and one much smaller spore (*b*, *b'*). Others, again, produced four spores all of different sizes (*c*). Some basidia produced three spores only. The three spores so produced were usually equal in size (*d*), but occasionally they were all of different sizes (*e*). Why one spore out of four should commence its development and then become aborted, as shown at *b* and *b'*, it is difficult to explain. Possibly this and the other irregularities which have been described are associated with irregular divisions or movements of the nuclei in the basidium-bodies.

A precocious excretion of a very large drop of water from the hilum of a young spore was described in Volume II.[1]

Remarks on Lepiota procera.—It may be asked : is the organisation of the hymenium of *Lepiota cepaestipes*, with which we have just become acquainted, common to all species of Lepiota ? The answer is : no. However, the only other Lepiota which I have examined for hymenial organisation is *L. procera*.

The magnificent fruit-bodies of *Lepiota procera*, the Parasol Fungus (Figs. 14 and 15 ; also Vol. I, Figs. 14 and 15, pp. 44 and 45), as every mycologist knows, stand upright for several days after their pilei have opened out. After some too limited observations of my own had revealed that the spore-discharge period of this species is at least three days in length, Mr. Sidney Dickinson, at my request, determined the full length of the spore-discharge period of a fruit-body growing in the open in Kew Gardens. The following is a summary of Mr. Dickinson's field-notes.[2] A very young fruit-body of *Lepiota procera* was first seen coming up on September 9, 1922. On September 10, 11, and 12, its pileus was found to be increasing in size. On September 13, the pileus was expanding and the *velum partiale* had become broken leaving an *annulus* around the stipe. A glass slide was then placed under the gills. At 10 A.M. the next morning, September 14, spores were found deposited on the slide. Thereupon a new slide was placed under the gills ; and, thereafter, this replacement was repeated daily until the spore-discharge period had come to an end. Spore-deposits were found upon the slides each morning from September 14 to September 21 inclusive. It was thus proved that the spore-discharge period of the fruit-body was of eight days' duration.

As we have just seen, the fruit-body of *Lepiota procera*, after the expansion of the pileus, remains standing and continues to shed spores for some eight successive days. It is therefore persistent, and not ephemeral like that of *L. cepaestipes*. This difference between the two species in persistence is correlated, as we might expect, with a difference in hymenial structure.

[1] These *Researches*, vol. ii, 1922, pp. 17–18, Fig. 9.

[2] *In litt.* I here desire to express my thanks to Mr. Dickinson for making the observations and sending me his record of them.

In *Lepiota procera* the hymenium is organised in accordance with either the Inocybe or the Panaeolus Sub-type. Unfortu-

FIG. 14.—*Lepiota procera*. A fruit-body with a pileus just beginning to expand and separating from the annulus. Photographed in Yorkshire by A. E. Peck. Natural size.

nately, the opportunity which came to me for examining the fruit-body was too limited to allow me to come to a decision on this point. However, I clearly perceived that the basidia are monomorphic (Fig. 17, B and C), and not tetramorphic as in *L. cepaestipes* (*cf.* Fig. 9, p. 16), and that considerable numbers of basidia with full-grown spores stand close together on the same small area of the hymenium (Fig. 16). It also became evident (Fig. 17, A) that, unlike what one finds in *L. cepaestipes* (*cf.* Figs. 7, C, and 10, pp. 14 and 17), one generation of basidia waits before developing its spores until the spores of the previous generation have been shot away. Moreover, it was found that the paraphyses are not conspicuous structures forming a hymenial pavement, like those of *L. cepaestipes*, but that they are relatively small and inconspicuous, like those of *Panaeolus campanulatus* and *Psalliota campestris* (Fig. 17, C, *e*).

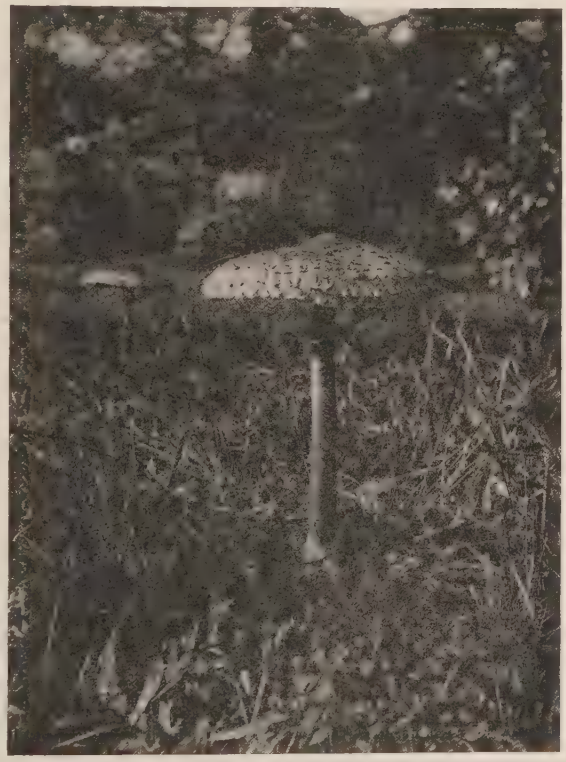


Fig. 15.—*Lepiota procera.* Two fully expanded fruit-bodies growing amid grass near trees. Photographed by J. E. Titley at Four Oaks, Warwickshire, England. The fruit-body in the fore-ground was 10 inches high.

If the spores of *Lepiota procera* became pigmented when ripening, like those of the species belonging to the Panaeolus Sub-type, the gills here and there doubtless would be more or less mottled, thus indicating an underlying organisation of the hymenium resembling

in all essentials that of the Panaeolus Sub-type. In Fig. 16, B, within the dotted contour-line, *a*, there is a group of basidia bearing spores only partially grown. Here, therefore, is an area corresponding to a light area on a gill of a Panaeolus. Surrounding this group of basidia are others bearing spores which are full-grown.

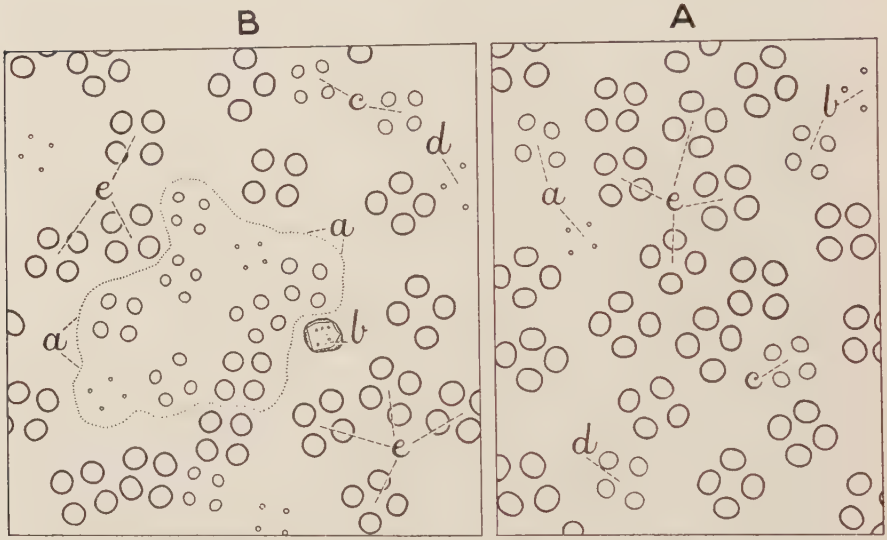


Fig. 16.—*Lepiota procera*. Two surface views of the hymenium, drawn with the *camera lucida*, showing the spores only. A : *a* and *b*, small groups or strings of basidia bearing partially grown spores ; *c* and *d*, isolated basidia bearing half-grown spores ; *e*, basidia bearing spores of full size. B : within the dotted area, *a a*, is a group of basidia bearing spores which have not yet attained full size ; *b*, a collapsed basidium which has discharged its spores ; *c d*, a string of basidia bearing partially grown spores ; *e*, basidia bearing spores of full size. The drawings afford evidence that the organisation of the hymenium of *Lepiota procera* differs greatly from that of *L. cepaestipes* and, in the main, resembles that of *Panaeolus campanulatus* and other representatives of the Panaeolus Sub-type. Magnification, 293.

Whether these spores have only just attained full size, or have long attained full size and are mature and about to be discharged, one cannot tell, since their walls do not develop any pigment ; but it is likely that, if they belonged to a Panaeolus, they would be more or less deeply pigmented and thus make up a dark gill-area. At A in Fig. 16 is another area in which basidia with partially-grown spores, *a*, *b*, *c*, and *d*, are scattered. Whether or not, if the spores became pigmented when maturing, this area would exhibit a certain amount of mottling, one cannot tell. The only means by which

one could definitely determine whether or not the underlying organisation of the hymenium of *Lepiota procera* resembles that of a Panaeolus in detail would be to watch the development of one and the same small area of the hymenium for several hours with the

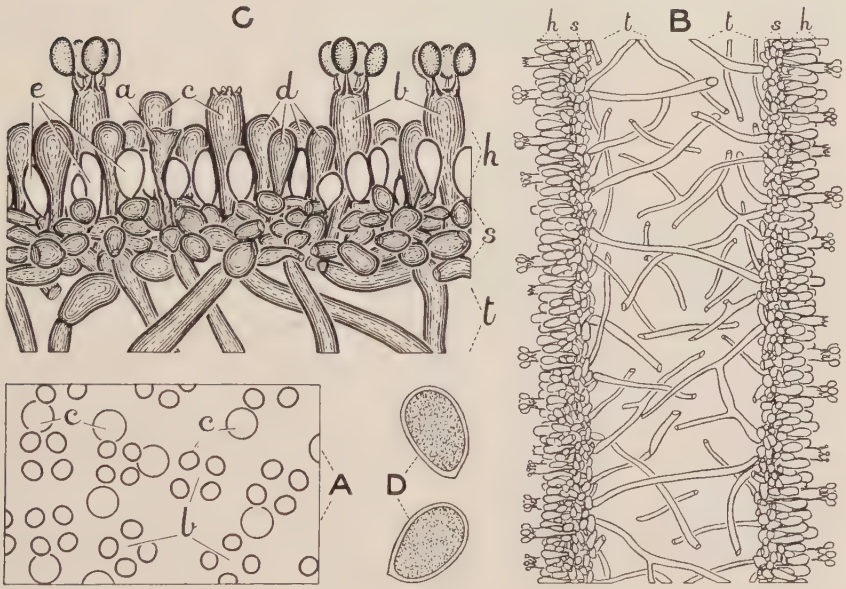


Fig. 17.—*Lepiota procera.* A, hymenium in a surface view showing spores on present-generation basidia, *b*, and prominent coming-generation basidia, *c*, which have not yet developed spores. B, vertical section through part of a gill showing: the hymenium, *h*, made up of basidia in various stages of development and of sterile paraphyses; the compact subhymenium, *s*; and the loose trama, *t*. C, a part of B more highly magnified: *h*, the hymenium; *s*, the subhymenium; and *t*, part of the trama; *a*, a collapsed past-generation basidium; *b*, present-generation basidia; *c*, coming-generation basidia; *d*, future-generation basidia; *e*, sterile paraphyses, left unshaded so that they may be readily distinguished from the basidia. D, two spores highly magnified, showing their thick walls. Magnification: A, about 290; B, 76; C, 293; D, 746.

microscope and observe the order in which the basidia produce and liberate their spores.

In the hymenium of *Lepiota procera*, in a general way, one can distinguish the following elements: past-generations-basidia (Fig. 17, *a* in C), present-generation basidia (*b* in C and A), coming-generation basidia (*c* in C and A), future-generations basidia (*d* in C), and paraphyses (*e* in C). The spores have unusually thick walls for colourless spores, and it is probable that, in correlation with this fact, the spores

develop slowly, several hours intervening between the first appearance of a spore on its sterigma and the moment of discharge. In a vertical transverse section through a gill (B) one can readily observe the hymenium (*h*), the subhymenium (*s*), and the trama (*t*). The trama is very loosely constructed and, in the general tendency of its hyphae to pass transversely from one side of a gill to the other, resembles that of *Lepiota cepaestipes* (*cf.* Fig. 17, B, and Fig. 9, p. 16).

The above observations show that the fruit-bodies of *Lepiota cepaestipes* and of *L. procera* differ very considerably in their arrangements for the production and liberation of spores. I cannot admit, therefore, that these two species are very closely related to one another genetically. Granting that they belong to the same genus, we must regard them as divergent types. In Saccardo's *Sylloge Fungorum*, up to and including the year 1912, there are descriptions of 374 species of Lepiota, and the genus has a very wide distribution.[1] In such a large genus it is not surprising to find that the organisation of the fruit-bodies for the production and liberation of spores is not perfectly uniform.

[1] P. A. Saccardo, *Sylloge Fungorum*, vols. v, ix, xi, xiv, xvi, xvii, and xxi.

CHAPTER II

PSATHYRELLA DISSEMINATA

Relations of *Lepiota cepaestipes* and *Psathyrella disseminata*—*Psathyrella disseminata*. Its Relation with Tree Stumps—The Structure and Functions of an Ozonium—Description of the Fruit-body—The Spore-discharge Period—The Gills—The Hymenium—Pilocystidia, Pleurocystidia, Cheilocystidia, and Caulocystidia—Correlation of Facts—Remarks on Taxonomy

Relations of Lepiota cepaestipes and Psathyrella disseminata.—*Psathyrella disseminata* is one of the two fungi upon which the Psathyrella Sub-type of fruit-body organisation is based. The other fungus, *Lepiota cepaestipes*, has just been described in detail. Both species are ephemeral, and both have a similar arrangement of the hymenium ; and, so far as the production and liberation of spores is concerned, they function in the same manner. However, the resemblance which they show in some of their structural and physiological characters does not prove that they are genetically closely related, for they differ greatly in a number of ways. Thus (1) the gills of the Psathyrella split from above downwards like those of many Coprini, whereas in the Lepiota the gills remain intact ; (2) the arrangement of the tramal cells in the gills of the Psathyrella is entirely different from that of the Lepiota ; (3) the spores of the Lepiota are colourless, whereas those of the Psathyrella are black ; (4) the stipe of the Lepiota is encircled by a well-developed annulus, whereas the stipe of the Psathyrella is perfectly bare ; and (5) the gills of the Lepiota are quite free from stipe, whereas those of the Psathyrella are adnate. It seems to me, therefore, that the two species have arrived at their similar arrangements for the rapid production and liberation of spores by convergence and not by direct descent from a common ancestor. Because two fungi show similar arrangements for the production and liberation of spores, that, in

itself, is not necessarily a proof of close relationship. For the purpose of building up a genealogical tree of the Hymenomycetes it is necessary to proceed along the old and safe lines, *i.e.* one must take into account *all* the characters which the species exhibit and not merely a few of them. Notwithstanding these remarks, we are fully justified in classing *Psathyrella disseminata* and *Lepiota cepaestipes* together from the physiological stand-point of the production and liberation of spores, for they carry out this one main function of their fruit-bodies in essentially the same manner. A parallel to the physiological mode of classification here adopted is to be found among the Phanerogamia in the division of the species into anemophilous, hydrophilous, and entomophilous groups, according to whether the wind, water, or insects are employed as agents in the cross-pollination of the flowers.

Psathyrella disseminata. Its Relation with Tree Stumps.—*Psathyrella disseminata* is one of the best-known of the Melanosporae, for it is frequently seen in late summer and autumn coming up in its favourite habitat upon and around old stumps of trees. The individual fruit-body is inconspicuous; for it has but a dingy grey appearance and is of small size, its stipe being rarely more than one and a half inches high, and its expanded pileus being, as a rule, only from one-third to one-half an inch in width. But the fruit-bodies are gregarious, and they make up for their sombre hue and small dimensions by their often multitudinous number. Sometimes, after rain, many hundreds of them—or even thousands—spring up overnight in one place, so that the phenomenon of their occurrence excites the astonishment of even the dullest observers.

A group of fruit-bodies coming up upon the soil around an inverted tree-stump is shown on a reduced scale in Fig. 18, and a small portion of the same group with the fruit-bodies of natural size and in their natural position is shown in Fig. 19. The stump had formed part of a healthy tree which was cut down about eighteen months before the photograph was taken. As soon as the tree had been felled, the stump was dug up, inverted, and set on bare soil under some trees, so that it might support ivy and become of ornamental value. Its destruction was soon begun by saprophytes of which one of the most successful was *Psathyrella dis-*

seminata. The vigour of the attack of the mycelium of this fungus

Fig. 18.—*Psathyrella disseminata*. Hundreds of fruit-bodies coming up from mycelium which has grown through stony soil from the base of an inverted tree-stump. The stump had been exposed for eighteen months. Photographed at Birmingham, England. About $\frac{1}{6}$ natural size.

is proved by the evidence of the numerous fruit-bodies shown in Fig. 18, all of which were produced at the expense of materials

withdrawn from the stump by the vegetating hyphae of the mycelium. By actual count, there are about 1,250 fruit-bodies included within the photograph ; but the fruit-bodies extended in a ring all around the stump. It therefore seems well within the mark to estimate their total number at between 2,000 and 3,000.

Fig. 19.—*Psathyrella disseminata.* Fruit-bodies coming up from mycelium which has grown through stony soil from the base of the inverted tree-stump shown in Fig. 18. Photographed *in situ* at Birmingham, England. Natural size.

Of especial interest is the fact that the fruit-bodies shown in Fig. 18 came up, not directly upon the stump but *upon the bare soil* around it. Most of the fruit-bodies were separated from the actual substance of the stump by a distance of several inches, and some of them by as much as a foot. Upon the soil adjacent to another similar stump, some of the fruit-bodies were found growing at a distance of not less than two feet from the stump's nearest point.

One naturally asks: what evidence is there that the fruit-bodies round about the stump shown in Fig. 18 were really produced at the expense of the stump? With the answer to this question we shall now concern ourselves. In the first place it must be remarked that the soil was stony, did not contain sticks or chips of wood, was not covered with turf, and had never been manured. Moreover, the fruit-bodies of *Psathyrella disseminata* came up upon it in the immediate vicinity of the stump and nowhere else. Reflecting on the non-nutritive character of the soil and upon the constant proximity of the fruit-bodies to the stump, I was led to the deduction that the mycelium of the fungus must stretch through the soil to the places of origin of the fruit-bodies. Direct observations soon proved that this deduction was correct, for it was found that the uppermost layer of soil down to a depth of about an inch contained a network of fine red mycelial cords and hyphae, which stretched radially away from the stump and spread itself throughout the whole region where the fruit-bodies were coming up. Furthermore, the bases of the stipes of the fruit-bodies were found to be in direct continuity with the mycelial cords.

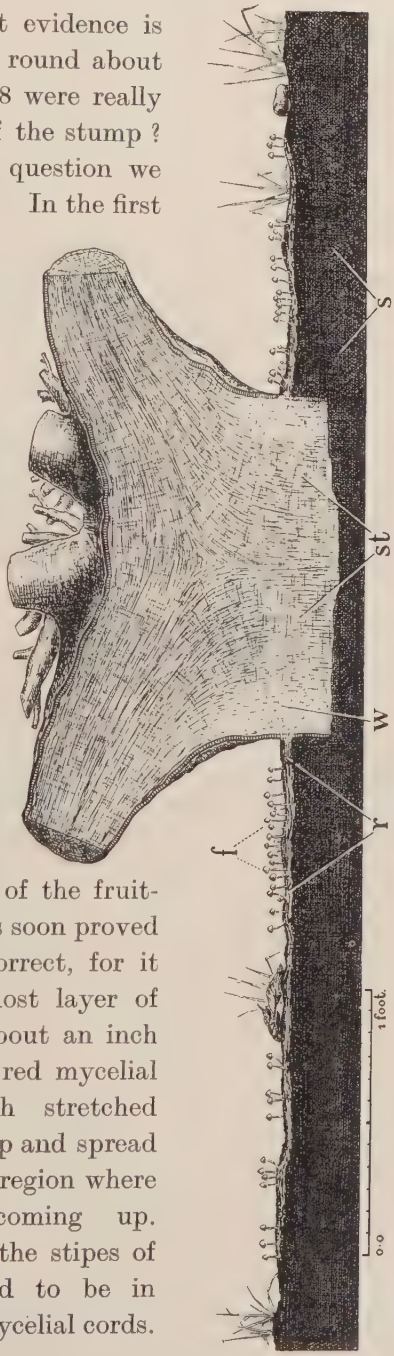


FIG. 20.—*Psathyrella disseminata*. Relations with substratum. *St*, an inverted stump of a tree which has been lying in position for eighteen months. The mycelium has vegetated in the stump at *w* and has produced a reddish mycelium or ozonium, *r*, which has penetrated through the soil, *s*, 1–2 feet from the stump. A few days before, a shower of rain had fallen and now the ozonium has given rise to a crop of numerous fruit-bodies, *f*. The magnification is shown by the scale.

The relation of the fruit-bodies of *Psathyrella disseminata* with one of the inverted stumps will be made clearer by reference to Figs. 20 and 21. In Fig. 20 is represented semi-diagrammatically a vertical section through the stump and soil. The wood at *w* was found to be very rotten, its destruction presumably having been caused by the mycelium of the fungus. This rotted wood was white and the mycelium within it colourless. The red mycelial cords and hyphae, to which reference has already been made, originated in crevices of the bark of the stump and stretched, as shown at *r*, through the interstices of the most superficial layer of the soil to a maximum distance of two feet. Upon this mycelium, and in continuity with it, the numerous fruit-bodies were situated. A small section of the soil with the mycelial network in the crevices is shown in Fig. 21 at A, and fruit-bodies in various stages of development are represented as arising from it. A small piece of a mycelial cord removed from the soil is shown at C with its natural size, and another piece at E highly magnified, while some of the individual hyphae which come off from the outer surface of the cords are represented at F.

The mycelial cords and the individual hyphae shown in Fig. 21 at A, C, E, and F, were all red and, in the damp black soil from which they were removed, much resembled fine roots ; but they anastomosed in all directions, so that the system which they composed was retiform. Their intimate contact with the particles of the soil was reminiscent of that of fine roots clothed with root-hairs. A single hypha, when branching, often produced a branch which ran parallel to, and became adherent to, the mother-hypha. Between a simple mycelial cord made up of two hyphae thus produced (F, *c*) and the largest cords which were about 0·3 mm. in diameter and consisted of some hundreds of hyphae (E) there were all transitional stages. The walls of the hyphae were thick (F) and it was owing to this fact that the mycelial cords, although so slender, were yet found to be tough and resistant to mechanical tearing.

So far as the stumps represented in Figs. 18 and 20 are concerned, the fruit-bodies were free ; for, as we have seen, they arose upon the red mycelium which penetrated through the soil. With other stumps, some of the fruit-bodies were found to be situated

directly on the bark free from the soil. It was observed that, where this was the case, the crevices of the bark were filled with a red mycelium which grew out to the exterior, as shown in Fig. 21, B and D, so as to form little red mycelial tufts. From such tufts the fruit-bodies eventually originated. The lower half of the stipe of one fruit-body is represented at B, *b*.

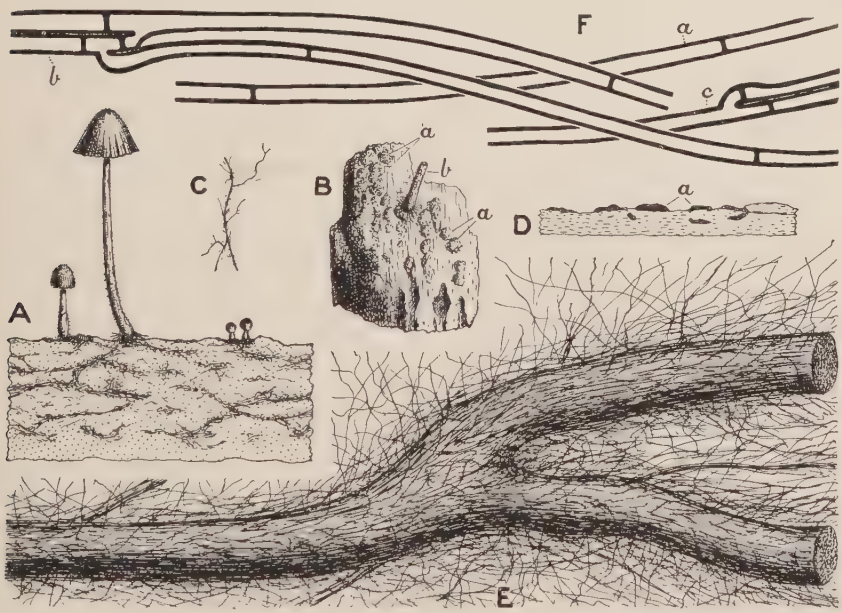


FIG. 21.—*Psathyrella disseminata*. Relations of ozonium with fruit-bodies. A, fruit-bodies attached to ozonium running through interstices of soil, B, surface view of a piece of bark with tufts of ozonium, *a* ; *b*, base of fruit-body. C, ozonium strands obtained from soil of A. D, cross-section of B showing ozonium tufts, *a*. E, part of the ozonium shown at C enlarged. F, ozonium hyphae from E ; *a*, a single hypha ; *b*, two hyphae in union ; *c*, a single hypha branching. A—D, natural size ; E, magnified 72 ; F, magnified 707.

The red mycelium under favourable circumstances, in addition to growing subterraneously in the manner which has just been described, can grow over the surface of the ground to a slight extent. A year after the observations represented by the photographs in Figs. 18 and 19 had been made, I found another inverted stump about which, up to that time, no fruit-bodies of *Psathyrella disseminata* had appeared. The soil around the stump was bare and also dry, for no rain had fallen for a considerable number of days.

I examined the surface of the soil carefully and discovered, to my surprise, that, within one or two feet of the stump, it was partially covered with red mycelium of *Psathyrella disseminata*. Reddish tufts of mycelium, like those in Fig. 21, B, *a* and D, *a*, looked upwards from the ground. I left the soil undisturbed. After some days had passed, there came a heavy rain-shower which thoroughly soaked the earth. The red mycelium at once revived and, about four days later, gave rise to an abundance of fruit-bodies gregariously crowded in the manner shown in Fig. 20.

The whole chain of events leading to this sudden and simultaneous production of thousands of fruit-bodies was doubtless as follows. The mycelium vegetated in the stump and, in moist weather, grew outwards from the stump for from one to two feet through the superficial layer of the soil. Within the soil, it developed into an anastomosing network of fine red cords and hyphae and, when thus developing, to some extent invaded the soil's exposed surface. The whole of the mycelium within and above the soil became laden with a certain amount of reserve food materials derived from the hyphae vegetating in the stump. Then came a spell of fine warm weather, in consequence of which the mycelium dried and became inactive. Then came a heavy down-pour of rain. The mycelium at once revived and, at the expense of its reserve food materials, within four days, produced a crop of fruit-bodies at the surface of the soil.

The Structure and Functions of an Ozonium.—The tough red mycelial network of hair-like cords and isolated hyphae which has just been described may be called an *ozonium*, for this name was originally given to mycelial systems of this character at a time when their connection with the fruit-bodies of various fungi had not yet been traced. An ozonium is now known to be produced by a number of Hymenomycetes. The commonest, which is reddish like that of *Psathyrella disseminata*, belongs to *Coprinus domesticus* and is frequently found beneath and upon the loosened bark of fallen Elm-trees.

An ozonium, such as that of *Psathyrella disseminata* or *Coprinus domesticus*, is not to be confused with the ordinary vegetative

mycelium. A fungus which produces an ozonium has its mycelium differentiated into two kinds. The first kind is the *ordinary vegetative mycelium*. This is composed of thin-walled colourless hyphae which, by means of enzymes which they excrete, actively attack the substratum, such as wood or bark. Thus the solid contents of the substratum are made available for the growth of the mycelium as a whole and, ultimately, for the production of fruit-bodies. The second kind of mycelium is the *ozonium*. This is developed at the expense of the ordinary vegetative mycelium and grows on the surface of, and away from, the nutritive substratum. Its hyphae are thick-walled and red, and are combined to form anastomosing cord-like strands. There is no evidence that it derives food-substances from the medium in which it grows, although it is doubtless able to absorb water. Its functions are two: (1) it serves to spread the fungus locally in a rapid manner, and (2) it enables the fruit-bodies to arise in more suitable locations than would often be possible, were these organs always obliged to arise directly upon the ordinary vegetative mycelium at the surface of the substratum.

One can easily conceive of the manner in which the ozonium of *Psathyrella disseminata* effects the local spread of the fungus. Let us suppose that one root of a stump has become infected with the mycelium. In a little while an ozonium is produced at the surface of the root and grows outwards through the soil. It is likely to meet with other roots of the same stump, give rise to ordinary hyphae which may penetrate them, and so cause their infection. One can further imagine that the fungus, by means of its ozonium, might even pass from one dead stump to another. Of course, in order to establish the correctness of these views, the support of direct experiments is required; and, unfortunately, up to the present, such experiments have not been undertaken. But the *rhizomorpha subterranea* of *Armillaria mellea* affords a parallel instance. Robert Hartig has shown that this structure, which is produced at the expense of the mycelium infecting the wood of the host-plant, can not only give rise to fruit-bodies, but can creep through the ten-centimetre-deep uppermost layer of the soil from one tree to another, penetrate the bark of new

roots with which it comes into contact, and thus spread infection locally.[1]

In *Coprinus domesticus* (= *C. radians*, Fig. 25, p. 43) we have an ozonium (*Ozonium auricomum* Link, *O. radians* Persoon)[2] which is very similar to that of *Psathyrella disseminata*. As already remarked, this ozonium is often to be found in the autumn under and upon the bark of fallen trees, particularly Elms. Owing to shrinkage of the wood and other causes, the bark of such trunks and logs often becomes loose. The ozonium flourishes in the space between bark and wood. One may suppose that: (1) not infrequently, the primary infection of a tree-trunk or log takes place at one spot; that (2) the ordinary vegetative mycelium then soon gives rise to an ozonium which rapidly makes its way in the open space under the bark; and that (3), as a result, the tree-trunk or log becomes infected at ever greater distances from the original seat of infection. I also suspect that the ozonium is able to grow through the soil in somewhat the same manner as that of *Psathyrella disseminata*.

The second function of the ozonium—that of enabling the fruit-bodies to arise in positions suitable for the successful production and liberation of spores—will now be discussed. So far as *Psathyrella disseminata* is concerned, a glance at Figs. 18, 19, and 20, will show the great advantage there was in the fruit-bodies arising not directly on the stump itself, but upon the ozonium penetrating through the soil. The two or three thousand fruit-bodies, had they

[1] Robert Hartig, *Wichtige Krankheiten der Waldbäume*, Berlin, 1874, pp. 27–29; also *Lehrbuch der Pflanzenkrankheiten*, Berlin, 1900, pp. 189–190, and Fig. 185.

[2] By Persoon, Link, and other botanists of a century ago the ozonium of *Coprinus domesticus*, like the rhizomorpha subterranea of *Armillaria mellea*, was believed to be an independent species of fungus. In 1888, Quélet (*Flore Mycologique de la France*, Paris, vol. i, 1888, p. 48) asserted that it is only the mycelial condition of *Coprinus domesticus* (his *C. radians*). Then, in 1901, Plowright ("Ozonium auricomum Link," *Trans. Brit. Myc. Soc.*, vol. i, 1901, pp. 181–182) confirmed this view by observing that the ozonium which had grown from a block of wood into the basal hole of a flower-pot containing an *Aspidistra* produced fruit-bodies of *Coprinus domesticus* at the top of the soil. Finally, in 1914, the connection of *Ozonium auricomum* and *Coprinus domesticus* was clearly demonstrated by F. T. Brooks ("Observations on pure cultures of some Ascomycetes and Basidiomycetes," *Trans. Brit. Myc. Soc.*, vol. iv, 1914, pp. 247–248) who germinated the spores of the Coprinus on sterilised blocks of Elm wood and observed that the mycelium thus produced developed into typical tawny strands of the ozonium.

all arisen directly on the sides of the inverted stump at and just above the surface of the ground, would have been very much crowded, and a large number of them would not have been able to shed their spores with any good chance of their being disseminated by the wind. As it was, the fruit-bodies arose at the surface of the soil upon the subterranean ozonium. In consequence of this, the

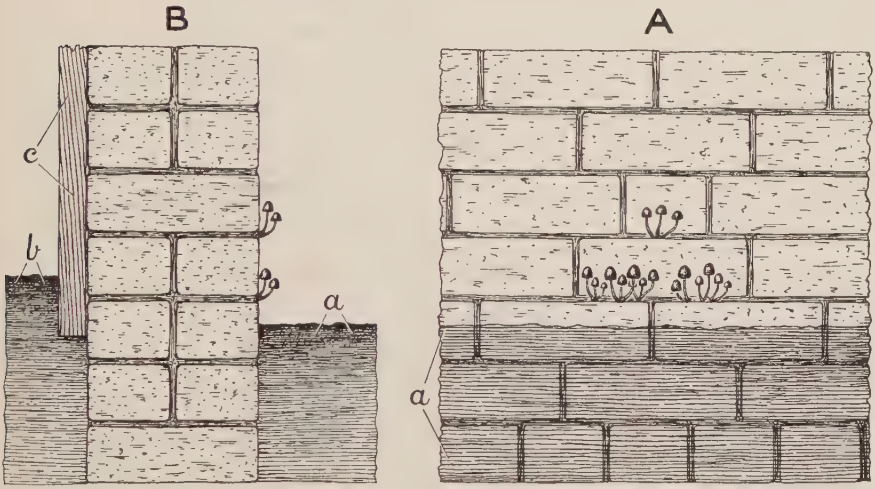


FIG. 22.—*Psathyrella disseminata.* Some fruit-bodies springing from the mortar of a brick wall of a green-house at the Priory, Kew. A, sketch showing the outside of the wall: *a*, bricks covered by garden soil. B, a section through the wall: *a*, garden soil; *b*, soil in green-house; *c*, wood-work in green-house, in which, presumably, the mycelium of the fungus was growing. The secondary mycelium or *ozonium*, doubtless, penetrated the wall *via* crevices in the mortar and then gave rise to the fruit-bodies in the position shown. $\frac{1}{10}$ natural size.

space for the development of the numerous fruit-bodies was greatly extended. Further, the spore-producing pilei were removed to a small distance from the sides of the stump, and thus the wind was given a better chance to carry off the spores. Evidently, although fruit-bodies can be, and often are, developed directly on the surface of the bark or wood of stumps, the production by the fungus of soil-penetrating ozonium gives greater freedom in the choice of the positions of origin of the fruit-bodies and, consequently, brings an advantage to the species so far as the dispersion of the spores is concerned.

The fruit-bodies of *Psathyrella disseminata* sometimes occur in

very curious and unexpected places. Thus I observed some of them on the outside of a green-house wall springing directly from

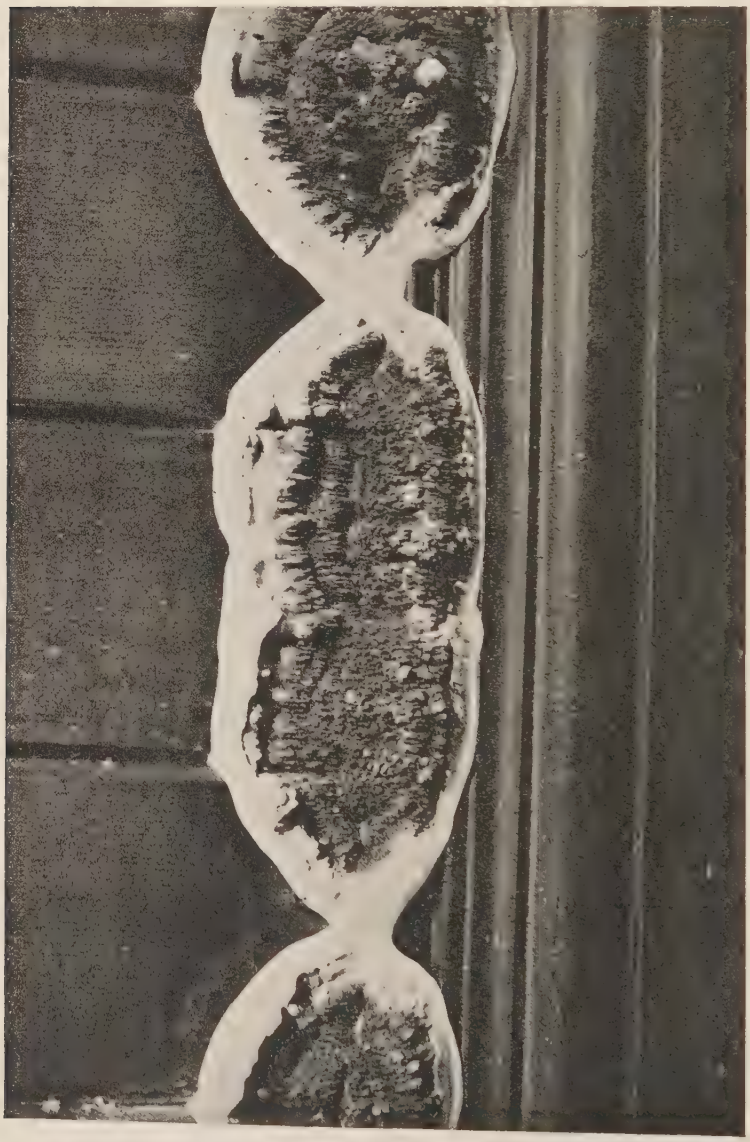


FIG. 23.—*Meruliius lacrymans*. Some fruit-bodies just above the wainscot in a billiard-room at Edgbaston, Birmingham, England. The mycelium was rotting a beam which is out of view Some of the mycelial cords made their way between the panels and formed the fruit-bodies over the varnished surface of the wood-work. The dark central portions of the fruit-bodies are roughened by shallow depressions covered by the hymenium. The white margins of the fruit-bodies are sterile, but below they have become darkened by the reddish-rusty spores which have fallen upon them. Photographed by the late W. Hillhouse in the presence of the author. $\frac{1}{2}$ natural size.

the mortar composing the two lowest horizontal joints between the bricks (Fig. 22, A). Opposite to these fruit-bodies inside the green-

house there was soil and a wooden stagework (B). Although I was unable to investigate the matter in detail, I have little doubt that the ordinary vegetative mycelium of the fungus had been attacking some wood present in the green-house, and that an ozonium had been produced which had penetrated through crevices in the mortar of the joints of the wall and had thus placed itself in an advantageous position for the production of the fruit-bodies. In support of this view, it may be mentioned that on two occasions I have observed the mycelium of *Merulius lacrymans*, the Dry-Rot Fungus (Fig. 23), passing through a brick wall *via* minute cracks and spaces in the mortar. In one instance, the mycelium had rotted the wood of a large beam at the top of a cellar wall. From the beam it spread downwards in a fanlike manner over the surface of the whitewashed wall on one side. Then, at a distance of nine inches from the beam, it made its way through the mortar of a horizontal joint, and thus passed to the other side of the wall where its fanlike growth over the bricks was continued. In the second instance, the Dry-Rot Fungus had attacked a wooden cupboard at one end of a very damp cellar, and the woodwork was enveloped with the fleecy mycelium (Fig. 24, A) and a few patches of fruit-bodies. At a distance of about a yard from the cupboard and a yard from the ground, there was a round hole in one of the joints of the mortar. The hole was only about 2 mm. in diameter, but through it there passed a mycelial cord to which was attached a mass of fleecy hyphae which spread out on the bricks so as to form a circular patch about six inches in diameter (Fig. 24, B). The builder, who took me to see this cellar, was firmly convinced that the fungus was living at the expense of the bricks and mortar; but, doubtless, if the wall could have been pulled down, it would have been found that the mycelium from the cupboard had made its way into the centre of the wall and had passed through crevices between the two courses of bricks until the little hole through which it emerged had been reached, the whole of the mycelial growth having been made solely at the expense of the wooden cupboard a yard distant.

A few remarks may be added here in regard to the position of growth of the fruit-bodies of *Coprinus domesticus*, which my studies of the genus Coprinus have led me to believe is identical with

Coprinus radians (Fig. 25).[1] This fungus has been observed with its fruit-bodies situated on damp plaster in old houses. We must not assume, however, that the mycelium in such a case feeds and grows at the expense of the plaster. It seems much more reasonable

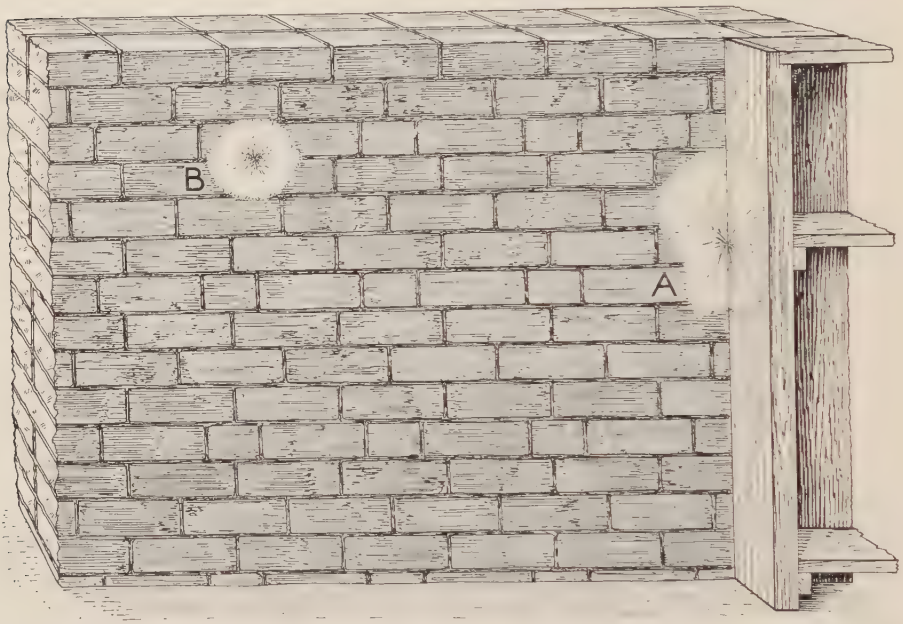


FIG. 24.—The lower half of a cellar-wall of a house at Birmingham, England. On the right, some wood-work attacked by *Merulius lacrymans*, the Dry-Rot Fungus. At A the mycelium is growing outwards over one of the boards and over the bare bricks. Some of the mycelium, *via* crevices in the mortar, has grown from the wood-work into the wall, has found its way between the two courses of bricks, and has finally emerged at B through a channel, about 2mm. wide, in the mortar of one of the joints. The patch of mycelium at B is 3 feet from the wood-work and is spreading radially over the surface of the wall.

to suppose that the mycelium attacks the moist wooden laths beneath the plaster first, and then produces an ozonium which penetrates through crevices in the plaster to the exterior surface, there giving rise to the fruit-bodies. The latter, thus becoming freely exposed on the exterior surface, are in a position where they can successfully produce and liberate their spores. The advantage of the existence of an ozonium in such a case is self-evident. Upon

[1] *Coprinus domesticus* was described before *C. radians* and alone appears in Fries' *Systema Mycologicum* (1821).

the tops of some very large upturned stumps of trees in a rubbish-yard in Queen's Cottage Grounds at Kew, I found a number of fruit-bodies of *Coprinus domesticus*. Some of them were arising on the bark, but others directly on the soil which was still filling the hollows and clefts between the roots (Fig. 25). An ozonium was found attached to the bases of the stipes, but it could be traced only a very short distance down into the soil. However, I have little doubt that it had originated from the substance of the roots, had grown upwards through the soil, and had given rise to the fruit-bodies at the soil's surface. Its existence had permitted the fungus to produce its fruit-bodies in situations which otherwise could not have been occupied.

Fig. 25.—*Coprinus domesticus*. Two fruit-bodies coming up on soil covering a large inverted stump of an Elm (Ulmus) in Queen's Cottage Grounds, Kew Gardens. Probably they were connected by an ozonium with the buried wood. Photographed by Miss E. M. Wakefield and the writer, 1912. Natural size.

In concluding this Section, it may be mentioned that an ozonium from the stump illustrated in Fig. 20 (p. 33) gave rise to fruit-bodies of *Psathyrella disseminata* during three successive summers. Moreover, the ozonium of a single stump may produce more than one crop of fruit-bodies in a single summer, for one of the stumps was observed to yield two crops several weeks apart. Each of these two crops developed a few days after rain had brought a dry spell of weather to a close. Whether or not the same piece of ozonium ever produces fruit-bodies more than once,

however, is uncertain and remains to be decided by future observation.

Description of the Fruit-body.—The very young pileus of *Psathyrella disseminata* is ovate and pale buff. It is bounded above by a firm palisade layer made up of cells which are rounded when seen from above (Fig. 26, B ; Fig. 27, A, *a*), and oval when seen in a transverse section through the pileus-flesh (Fig. 27, B, *a*). Among the palisade cells are special cells which are produced outwards into long slender unicellular hairs or *pilocystidia*, each having a swollen base and a simple cylindrical shaft (Fig. 26, A and B ; Fig. 27 : A, *d* ; B, *d* and *e*). Each pileus, as in *Coprinus curtus* or *C. ephemerus*, bears several hundreds of these hairs. In addition, on the top and sides of the pileus above the palisade layer there lie loose or easily detachable a number of more or less spherical cells resembling those of certain Coprini, *e.g. C. stercorarius*, *C. niveus*, and *C. curtus* (Fig. 27, A, *b* ; B, *b*). Many of these cells, as they grow older, become brown and their walls become decorated with small crystals of calcium oxalate (Fig. 27, A, *c* ; B, *c*). The numerous hairs and the loose spherical cells give the pileus a roughened appearance. Hence, the pileus has been called *scurfy* by Massee [1] and *furfuraceous* by Patouillard.[2] When a young fruit-body, well supplied with water below, is placed in air saturated with water-vapour, the tops of the hairs rapidly excrete drops of a slimy fluid (Fig. 27, *d* and *e*). Knoll, who regards the hairs as trichome-hydathodes,[3] has discovered that the drops, although soluble in

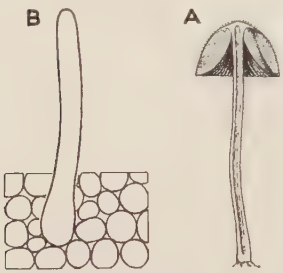


FIG. 26.—*Psathyrella disseminata*. A, vertical section through a fruit-body. The gills are not mottled but evenly black. The stipe is hollow, and the pileus bears hairs on its exterior. B, a surface view of a part of a pileus showing rounded cells and one of the hairs. A, natural size ; B, magnification, 220.

[1] G. Massee, *British Fungus-Flora*, London, 1892, vol. i, p. 345.

[2] N. Patouillard, *Tabulae analyticae fungorum*, Paris, 1883–1886, No. 351, p. 160.

[3] F. Knoll, "Untersuchungen über den Bau und die Function der Cystiden und verwandter Organe," *Jahrb. für wiss. Bot.*, Bd. 50, 1912, pp. 457–468, 490.

water, persist in 95 per cent. alcohol.[1] The excretion, according to this observer, is of a colloidal nature.

The pileus, on expanding, becomes campanulate and then soon begins to discharge its spores. Its diameter in the fully expanded condition varies from 1 to 1·5 cm. Whilst the expansion is taking place, the colour of the pileus changes from whitish or pale buff to grey, except at the disc which becomes yellowish. The yellow colouring matter of the disc is deposited in certain of the projecting hair-cells. The cells which make up the firm outer palisade layer of the pileus grow rapidly in size during the expansion of the pileus and, in consequence, the hairs situated between them become correspondingly more scattered and less noticeable. The loose spherical cells also tend to disappear from the pileus. In Massee's description the pileus is said to be "at first scurfy, then naked." For field purposes this may be taken as true, although careful search reveals some prominent or collapsed hairs on the surface of the pileus to the very last. The pileus, whilst becoming campanulate, splits radially from above downwards along the lines of the gills, more particularly along those of the larger ones. It thus becomes plicate in the same manner as the pileus of *Coprinus plicatilis* or *C. curtus*. A cleft gill in cross-section resembles the shape of the letter Y. The radial cleavages, which are about thirty in number altogether, permit of the rapid expansion of the pileus without any corresponding growth of the pileus-flesh; and, indirectly, they also serve to bring into existence suitable interlamellar spaces for the discharge of the spores. The flesh of the pileus is thickest at the disc where, by suitable growth-movements, it serves the purpose of opening out the pileus. The flesh on the sides of the pileus is extremely thin; and, although when the pileus is very young and ovate it forms a continuous sheet, yet when the pileus opens out it becomes split into a series of radial ribbons, each ribbon supporting one-half of each of two adjacent long gills and often, in addition, the whole of a very short gill.[2]

[1] *Loc. cit.*, pp. 463–464.

[2] It is a mistake to suppose that there is no pileus-flesh on the sides of the pilei of *Psathyrella disseminata*, *Coprinus plicatilis*, etc. Gills are projections of the flesh, which extend the area on which the hymenium may be developed. If, in either of these two fungi, the gills were removed from the young and unexpanded pileus, we

The gills (Fig. 26, A, p. 44) are very thin, 5 to 7 mm. in length and 2 to 3 mm. in width, very acutely wedge-shaped in cross-section

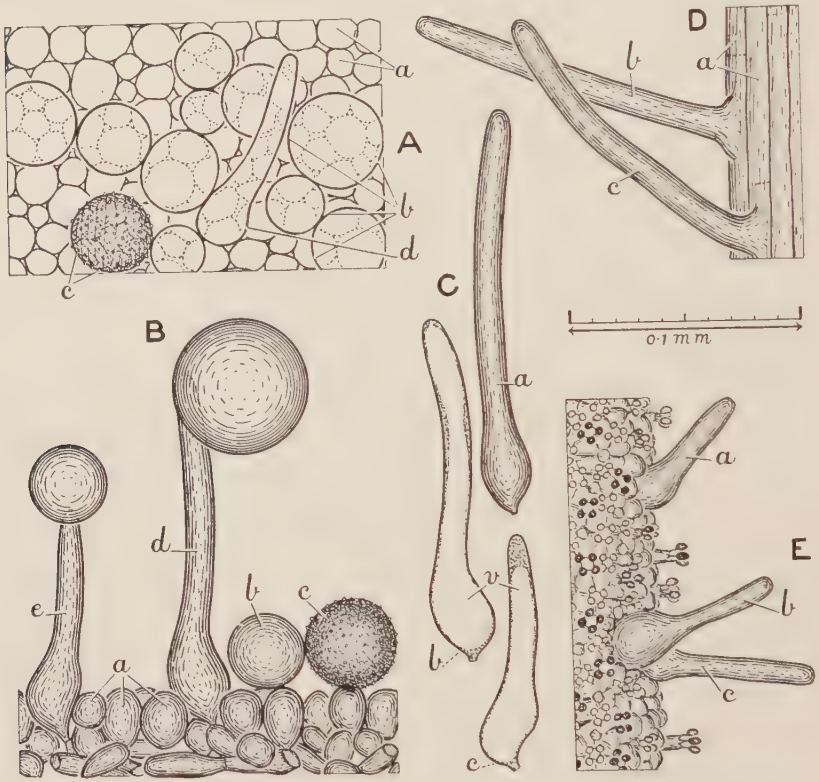


FIG. 27.—*Psathyrella disseminata.* A, surface view of part of the top of a pileus: *a*, the palisade cells; *b*, loose, colourless, spherical cells resting on the palisade cells; *c*, a loose cell which has become brown and now has crystals of calcium oxalate on its wall; *d*, a pilocystidium. B, a vertical section through the top of a pileus: *a*, the palisade cells; *b*, a loose colourless spherical cell resting on the palisade cells; *c*, a loose brown cell with crystals of calcium oxalate on its wall; *d*, a long pilocystidium with a mucilaginous drop of fluid excreted laterally at its apex; *e*, a shorter pilocystidium with a smaller drop excreted at its apex. C, pilocystidia: *a*, the longest observed; *b* and *c*, two others showing their protoplasmic contents enclosing the vacuoles *v*. D, part of the top of a stipe: *a*, outer cells; *b* and *c*, two caulocystidia. E, part of the free edge of a gill near the periphery of the pileus: *a*, *b*, *c*, three cheilocystidia. Magnification, 300.

when very young, and adnate to the stipe. In my specimens they had a greyish-violet tinge, but Massee and Patouillard both speak

should have the flesh left by itself in the form of a very thin and ovate membrane. That this membrane actually becomes split into ribbons radially, as the pileus expands, must not be used as an argument for its non-existence.

of them as being at first grey and finally black. The surface of the gills is even and without the slightest trace of mottling (Fig. 26, A). This is due to the fact, which will be explained more fully a little later, that the basidia develop their spores in the same manner as *Lepiota cepaestipes*. In the entire absence of mottling from the gills, *Psathyrella disseminata* differs from all the species of Panaeolus and Anellaria. Cystidia are absent from the lateral surfaces of the gills, but are present along the free gill-margins at the periphery of the pileus where they extend centripetally for a short distance only (Fig. 27, E, *a*, *b*, *c*).

The stipe varies in length from about 2·5 to 4 cm. It is thin, for it is not much more than 1 mm. thick, even, tapering upwards very slightly, whitish, weak, easily broken, frequently crooked when the fruit-bodies are congregated, and hollow (Fig. 26, A, p. 44). At its base it may give off radiating fibrils. The surface of the stipe, especially in its lower half, bears a considerable number of hairs which often occur several together in little groups (Fig. 21, A, p. 35). The hairs, which are unicellular, resemble those on the pileus and have inflated bases and cylindrical shafts (Fig. 27, D, *b* and *c*).

The spores are black in the mass on white paper but dark brown when examined with the microscope, smooth, oval, rounded at the end containing the apical pore and more pointed at the other end, 9 to 11 μ long, and 4 to 5 μ wide.

The Spore-discharge Period.—In order to determine the length of the spore-discharge period, I secured a clump of fruit-bodies which were just opening, together with the substratum upon which they were growing, and removed them to a room in a house where they were set in their natural upright position under a bell-jar. Glass slides were then placed under the pilei to collect any falling spores ; and, when one slide was removed for examination with the microscope, another was put in its place. Spores began to settle on the first glass slide at about 11 o'clock in the morning and continued to fall all day. At 11 P.M. a new slide was set under the pilei. Next morning at 9.30 A.M., the spore-deposit on the slide was very thin, and all the fruit-bodies had collapsed. These observations, although not so complete as is desirable, show that spore-discharge in *Psathyrella disseminata* takes place during a period of from 12 to

$22\frac{1}{2}$ hours. Probably the actual length of the period did not exceed 18 hours.

There is no doubt that the fruit-bodies of *Psathyrella disseminata* are very ephemeral. Every field mycologist knows that they die down within 24 hours of stretching their stipes and expanding their pilei. The short period of existence of the mature fruit-bodies is evidently strictly correlated with the brief duration of the spore-discharge period. A fruit-body of a fleshy Agaric is of no value whatever to the species to which it belongs when once it has shed its spores, and there would be nothing gained, and there would be a good deal lost, if it were provided with materials for prolonging its existence after its usefulness was over. As one might therefore expect, on the ground of economy, the gills of a fruit-body which has just shed its last spores are practically exhausted of their contents. The individual cells of the hymenium, subhymenium, and trama no longer contain any massive contents. The protoplasm, if present, is in the form of an extremely attenuated layer lining the cell-walls, and in each cell it surrounds one huge vacuole. It is no wonder that such an exhausted fruit-body soon dies, collapses, and becomes a prey to saprophytes. The death of the fleshy fruit-bodies of Hymenomycetes, almost immediately after spore-discharge has ceased, finds its parallel in the withering of the corolla of the Lady's Slipper Orchid very shortly after the pollination of the stigma has taken place, and also in the death of many insects very soon after their contribution to the propagation of the species has been made—the males after they have effected the fertilisation of the females, and the females after they have completed the laying of their eggs.

It is important to grasp the central fact of the ephemeral nature of the fruit-bodies of *Psathyrella disseminata*, for only by so doing shall we understand the delicate structure of the fruit-body as a whole and the organisation of the hymenium, which are strictly correlated with it.

The Gills.—The gills are acutely wedge-shaped in cross-section in the unexpanded fruit-body and they look downwards toward the earth when the spores are being liberated, in the manner shown for *Lepiota cepaestipes* in Fig. 5 (p. 12). Moreover, every part of

the hymenium produces and liberates spores at the same time. It is evident, therefore, that *Psathyrella disseminata* belongs to the Aequi-hymeniiferous Type. However, I have not yet tested the gills by experiment to find out whether or not they follow the usual rule and are positively geotropic : it is just possible that they are non-geotropic like those of the Coprini. The gills are developed radially in the young fruit-body. The stipe is strongly negatively geotropic and, just before spore-discharge is about to begin, whilst the pileus is expanding, bends upwards and so brings the median planes of the gills into approximately vertical positions. Since the individual gills are wedge-shaped in cross-section, their hymenial surfaces thus come to look more or less downwards. Further, as the pileus expands, the gills become cleft for a certain distance down their median planes, so that the cleft portion of each is shaped in cross-section like the letter V. The hymenium toward the top of each gill thus comes to look downwards to the earth at a considerable angle.

The Hymenium.—The rapid discharge of the spores, which as we have seen is accomplished within about 18 hours, is brought about in *Psathyrella disseminata* by an organisation of the hymenium that is similar to that in *Lepiota cepaestipes* : (1) there is a reduction of the number of generations of basidia to four ; (2) the four generations of basidia have differential protuberancy and are therefore tetramorphic ; (3) there is overlapping in time in the development of the sterigmata and spores in successive generations, so that the four generations of basidia ripen and discharge their spores in rapid succession ; and (4) the paraphyses are large and joined into a definite pavement-like system, so that they fill up the spaces which must necessarily be left between adjacent basidia if the latter are to develop and discharge their spores without mutual hindrance.

Illustrations of the hymenium are given in Figs. 28 and 29. In Fig. 28 at A there is shown a *camera-lucida* drawing of a piece of the hymenium, as seen in face view, in which only the spores and the exhausted basidia are represented. The first two generations of basidia, *ab*, have discharged their spores and only their collapsed bodies now remain. Which of these bodies belong to the first and which to the second generation one cannot now decide. The spores on the third-generation basidia, *c*, which are all darkly pigmented

and about to be discharged, are shown in uniform black. The spores of the fourth and last generation of basidia, *d*, which have already begun to be affected by the process of pigmentation, are lightly shaded. One is struck, when examining the hymenium as a whole, by the uniform nature of the development. From such an illustration as has just been described one can readily understand why it is that, to the naked eye, the sides of the gills never show the least trace of mottling

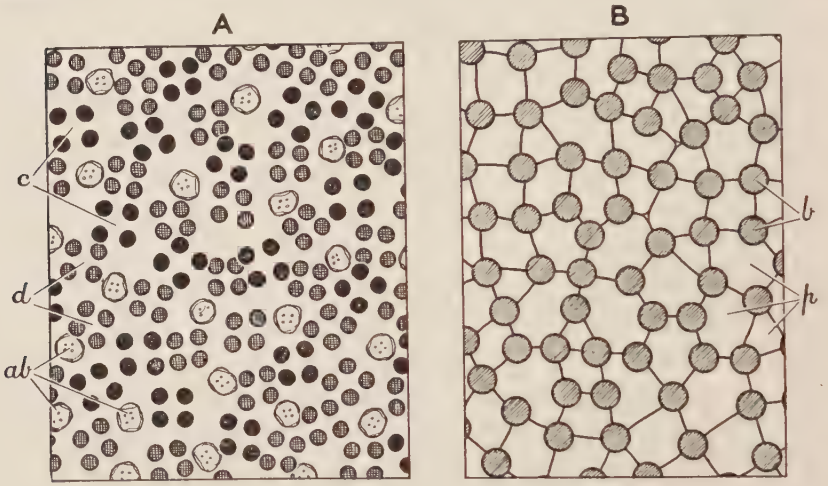


FIG. 28.—*Psathyrella disseminata*. Surface views of the hymenium from drawings made with the *camera lucida*. A shows: *ab*, the collapsed basidia of the first and second basidial generations; *c*, the ripe or almost ripe spores of the third basidial generation; and *d*, the ripening spores of the fourth basidial generation. B, a plan showing the relative positions of the basidia, *b*, and the paraphyses, *p*. Magnification, 586.

but appear to be uniformly grey (*cf.* Fig. 26, A, p. 44). In Fig. 28 at B are shown the well-developed paraphyses which are united into a system. They never produce spores and evidently function as sterile space-makers in that they keep adjacent basidia sufficiently far from one another to secure the proper freedom of the spores during their development and discharge.

The semi-diagrammatic cross-section represented in Fig. 29 will throw further light on the structure of the hymenium. The first generation of basidia, *a a a*, is the most protuberant, the second generation, *b b*, less protuberant, the third, *c c*, still less protuberant, and the fourth, *d d d*, so far as their bodies are concerned, not pro-

tuberant at all. It is clear that the basidia as a whole are tetramorphic. The overlapping in the time of development is obvious. The spores of the first-generation basidia are fully pigmented and, therefore, nearly ripe and about to be discharged. The spores of the second-generation basidia have not only already attained full size but are undergoing pigmentation. The spores of the third-generation basidia have attained full size but are still colourless.

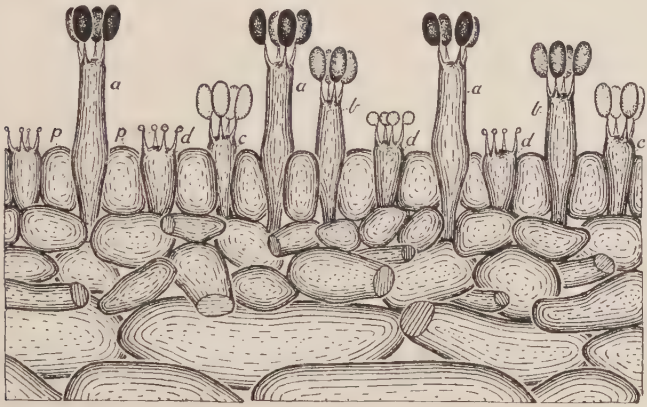


Fig. 29.—*Psathyrella disseminata.* Transverse section through the hymenium during its development. There are four generations of basidia: *a*, the first, bearing black spores; *b*, the second, bearing brown spores; *c*, the third, bearing fully-grown colourless spores; and *d*, the fourth, bearing rudiments of spores. *p*, a paraphysis. Magnification, 587.

The spores of the fourth-generation basidia are only just beginning to develop and have not yet attained to full size. This overlapping in the time of development of successive generations of basidia contrasts very strongly with what occurs in the hymenium of the Panaeolus Sub-type, for in *Panaeolus campanulatus*, *Stropharia semiglobata*, *Psalliota campestris*, etc., one generation of basidia (except of course the first) never begins to develop spores until the spores on the basidia of the preceding generation have been discharged. The paraphyses *p p* in Fig. 29 appear as oval or quadratic cells, and one can perceive that they are just about the right size to maintain sufficient space between adjacent spore-bearing basidia. At the same time it is evident that they support the basidia in their

upright positions. They may also assist the basidia by supplying them with water and possibly also with food-stuffs. The sub-hymenium consists of rounded or oval cells about two layers thick, whilst the trama is composed of elongated swollen oval cells with long axes directed perpendicularly to the surface of the pileus.

Previous authors have overlooked the polymorphism of the basidia which has just been described. Patouillard merely remarks that the basidia are protuberant; but, as we have seen, this is only true of some of them and not of all. His illustration of the hymenium shows a single protuberant basidium surrounded by paraphyses.[1] Boudier, in his *Icones*, has a sketch of a cross-section of the hymenium in which are represented five paraphyses and two highly protuberant basidia.[2] Doubtless, like Patouillard, he only noticed the most prominent basidia and failed to observe that the basidia, taken all together, have four different lengths.

Just as in *Lepiota cepaestipes*, there are no cystidia on the sides of the gills. Cystidia are also absent from the free gill-edges except for a very short distance from the periphery of the pileus. The hairs, which have already been noticed as occurring on the top of the pileus, are present at the rim of the pileus and appear to pass down below the pileus so as to make a slight invasion of the gill-margins. The best way to detect these marginal hairs is to take a whole pileus from its stipe, invert it, and examine it with the low power of the microscope. It will then be seen that there are basidia on the gill-edges but that there are a few hairs also present on these edges near the pileus-rim (Fig. 27, E, p. 46). These hairs resemble in structure those on the pileus and on the stipe, *i.e.* they are unicellular and have swollen bases and long cylindrical shafts.

Pilocystidia, Pleurocystidia, Cheilocystidia, and Caulocystidia.—It seems to me that there is room for reform in the nomenclature of those hair-like cells which so often occur on the sides of the gills, on the free gill-margins, on the top of the pileus, and sometimes also on the stipes of many Agaricineae. We might extend the meaning

[1] N. Patouillard, *Tabulae analyticae fungorum*, Paris, 1883–1886, No. 351, p. 160.

[2] E. Boudier, *Icones Mycologicae ou Iconographie des Champignons de France principalement Discomycètes*, Paris, 1905–1910, Tome i, No. 85, Pl. 140.

of the term *cystidium* and define it as meaning *any hair-like cell which is not a basidium or a paraphysis and which freely projects from the cell-layer that covers the top of the pileus, the sides or edges of the gills, or the stipe.* Then we could speak of such hairs as occur on the top of the pileus of *Psathyrella disseminata* as *pilocystidia* (Fig. 27, A and B, p. 46), of such hairs as occur on the gill-edges as *cheilocystidia* (E), and of such as occur on the stipe as *caulocystidia* (D). In *Psathyrella disseminata*, as it happens, there are no cystidia on the sides of the gills ; but, if there were, we could call them *pleurocystidia*. This nomenclature can be used generally for Agaricineae, and has the advantage of retaining the old term *cystidium* which has now been used in connection with the gills for nearly one hundred years. The prefixes are topographical only. The use of these terms might save words in systematic descriptions and introduce into them a little greater precision.

A still better set of terms, if the history of mycological nomenclature did not exist, would be *pilotrichome, pleurotrichome, cheilotrichome,* and *caulotrichome* ; but if they were to be adopted the term *cystidium*, which has become engrained in the literature of the Hymenomycetes, would be dropped. Doubtless, few mycologists would be willing to start *de novo* in this way. The best compromise therefore seems to me to be the acceptance of the terms *pilocystidium, cheilocystidium, pleurocystidium* and *caulocystidium.*

Correlation of Facts.—The fruit-body of *Psathyrella disseminata* is organised for a very ephemeral existence only. Its spore-discharge period, as we have seen, is not longer than about 18 hours. The stipe is very slender, hollow, and weak ; and the pileus is very delicate. The pileus-flesh is well-developed only at the disc ; elsewhere, it is extremely thin and becomes split up into radial ribbons during the expansion of the pileus. The gills are very thin and become partially split down their median planes : they are evidently too frail to function for any long period. In the general softness of its mechanical structure and in its extremely limited power of resisting loss of water by transpiration, the fruit-body of *Psathyrella disseminata* resembles the fruit-bodies of many small and very ephemeral Coprini such as *Coprinus ephemerus.*

Correlated with the very short spore-discharge period, the number

of generations of basidia is limited here, as in *Lepiota cepaestipes*, to four. The production and liberation of the spores of these four generations is hastened, in contrast with what happens in the Panaeolus Sub-type, by an overlapping in the times of the development of the four sets of basidia. Thus, the second generation of basidia is only a slight developmental stage behind the first, the third generation only a slight developmental stage behind the second, and the fourth generation of basidia only a slight developmental stage behind the third. Correlated with this developmental overlapping, the four generations of basidia are tetramorphic : the bodies of the first-generation basidia protrude far beyond the general level of the hymenium ; those of the second-generation basidia are less protuberant ; those of the third-generation basidia are still less protuberant ; whilst those of the fourth-generation basidia are not protuberant at all. This tetramorphism permits of a greater crowding of the basidia on any given area of the hymenium, and therefore a greater production of spores, than would be possible with monomorphism, such as we have in the Panaeolus Sub-type ; and, at the same time, vertical contact between the spores of any adjacent basidia belonging to different generations is prevented. Lateral contact between the spores of any two adjacent basidia is prevented by the presence between the basidium-bodies of large and well-developed permanently sterile paraphyses, which are necessary as space-makers. The four generations of basidia together with the paraphyses form a beautiful mosaic-work of such a kind that the spores on adjacent basidia cannot mutually interfere with one another, either vertically or horizontally, during either their development or discharge ; while, at the same time, any given area of the hymenium is employed so as to produce the maximum number of spores which can be liberated in the very short spore-discharge period.

Remarks on Taxonomy.—Quélet, in 1888, removed *Psathyrella disseminata* from the genus Psathyrella in which Fries had placed it, and included it in the genus Coprinus.[1] It thus came to have the name *Coprinus disseminatus* (Pers.) Quél. Lange, in 1915,

[1] L. Quélet, *Flore Mycologique de la France et des Pays Limitrophes*, Paris, 1888, p. 42.

published a monograph on the genus Coprinus in which he described in detail thirty-three species found in Denmark. In respect to *Psathyrella disseminata*, he followed Quélet, for he described this fungus as a Coprinus and placed it in his section Nudi.[1]

I have no doubt whatever that Quélet and Lange are in error in placing *Psathyrella disseminata* in the genus Coprinus and have so expressed myself in another place.[2] My reasons for holding this view will now be given in as concise a form as possible.

The chief points in connection with the mechanism for the production and liberation of spores in the genus Coprinus, *i.e.* in the Inaequi-hymeniiferous Type,[3] may be summarised as follows :

(1) The gills are very thin,
(2) The gills are parallel-sided or subparallel-sided,
(3) The gills are not positively geotropic,
(4) Usually the hymenium on one side of a gill at maturity looks slightly downwards and that on the other side slightly upwards,
(5) The spores ripen in succession from below upwards on each gill,
(6) The spores are discharged in succession from below upwards on each gill,
(7) Autodigestion proceeds from below upwards on each gill and removes those parts of the gills which become spore-free and which, if they continued in existence, would become mechanical hindrances to the fall of the remaining spores.

All the facts just given are correlated with one another. In the genus Coprinus the ripening and discharge of the spores from below upwards on each gill, and the autodigestion from below upwards on each gill, are special arrangements which permit of successful spore-discharge from parallel-sided non-geotropic gills.

[1] Jakob E. Lange, "Studies in the Agarics of Denmark," *Dansk Botanisk Arkiv udgivet af Dansk Botanisk Forening*, Part ii, No. 3, 1915, p. 46.

[2] A. H. R. Buller, "Some Critical Remarks on the Generic Positions of *Psathyra urticaecola* Berk. et Broome, *Coprinus plicatilis* Fr., and *Psathyrella disseminata* Pers.," *Transactions of the British Mycological Society*, vol. v, 1917, pp. 482–489.

[3] These points were discussed at length in vol. ii, 1922, ch. ix, and will be treated of in detail in the chapters devoted to a description of the Coprinus Sub-types.

By contrast, the chief points in connection with the mechanism for the production and liberation of spores in non-Coprinus Agaricineae, such as the species of Amanita, Psalliota, Panaeolus, etc., all of which belong to the Aequi-hymeniiferous Type, are as follows :

(1) The gills are sometimes very thin but are usually relatively thick,

(2) The gills are not parallel-sided but are more or less acutely wedge-shaped in cross-section,

(3) The gills are positively geotropic,

(4) Every part of the hymenium on any gill in a normally oriented fruit-body looks more or less downwards,

(5) The spores do not ripen in succession from below upwards on each gill but more or less uniformly all over the gill-surfaces,

(6) The spores are not discharged in succession from below upwards on each gill but more or less uniformly all over the gill-surfaces,

(7) Autodigestion does not proceed from below upwards on each gill or involve any part of a gill during the period of spore-discharge.

In the light of these criteria, let us now consider the generic position of *Psathyrella disseminata*.

If one examines the gills carefully with the help of the microscope, one finds that they satisfy the seven criteria for a non-Coprinus lamellate fungus, possibly with the exception of the third. The gills are (1) thin, but (2) they are acutely wedge-shaped in cross-section. Whether or not they are (3) positively geotropic I do not know. However, (4) every part of the hymenium on any gill in a normally oriented fruit-body looks more or less downwards ; for the stipe, by means of a very precise negatively geotropic reaction, brings the median planes of the gills into approximately vertical positions. The most important criteria are the fifth, sixth, and seventh. The spores (5) do not ripen in succession from below upwards on each gill but more or less uniformly all over the gill-surfaces. The spores (6) are not discharged in succession from below upwards on each gill but more or less uniformly all over the

gill-surfaces. Finally (7), autodigestion does not proceed from below upwards on each gill or involve any part of a gill during the period of spore-discharge. The collapse of the gills and pileus as a whole takes place only when spore-discharge has ceased. *Psathyrella disseminata* is therefore not a Coprinus ; and, for the present at least, there is no good reason for changing its generic position from that given to it by Fries.

Quélet[1] has also transferred *Psathyrella impatiens* Fr. and *Psathyrella hiascens* Fr. to the genus Coprinus and, in respect to the first-named, has been followed by Lange.[2] Unfortunately, I have not yet been able to examine these species myself ; but I cannot help feeling that at present there is no justification for regarding them as Coprini. Fries[3] makes the comment that *Psathyrella hiascens*, while having the habit of a Coprinus of the Veliformes section, differs greatly from Coprini in its somewhat stiff and dry gills. Dry gills certainly do not suggest a Coprinus. It will be necessary to re-examine the two species here under discussion and to apply to them the seven criteria which have been enumerated above. I am inclined to think that, when this has been done, the view of Fries, rather than that of Quélet or Lange, will be upheld.

There can be no doubt that some of the smaller Psathyrellae, such as *Psathyrella disseminata* and *P. hiascens*, when examined in the field, have a certain resemblance to some of the smaller Coprini ; and Fries, Quélet, Lange, Atkinson,[4] and others have rightly recognised this fact. These resemblances are : (1) the ephemeral nature of the fruit-bodies, (2) the softness of the pilei, (3) the weakness and hollow structure of the stipe, (4) the extreme reduction of the pileus-flesh, (5) the splitting of the flesh and gills along radial lines so that the pileus becomes furrowed when it expands, (6) the black spores, and (7) the change of colour of the pileus from whitish or yellowish to dingy grey during the ripening of the spores, which can be seen from above owing to the translucency and splitting of the flesh and gills. The field characters in which the

[1] E. Quélet, *loc. cit.*

[2] J. E. Lange, *loc. cit.*, p. 47.

[3] Elias Fries, *Hymenomycetes Europaei*, Upsaliae, 1874, p. 314.

[4] G. F. Atkinson (*Studies of American Fungi, Mushrooms edible, poisonous, etc.*, Ithaca, U.S.A., edition 2, 1901, p. 48) states that the fruit-bodies of *Psathyrella disseminata* " resemble small specimens of a Coprinus."

smaller and hitherto doubtful Psathyrellae differ from the smaller Coprini are as follows : (1) the gills do not undergo autodigestion from below upwards during the period of spore-discharge even in the slightest degree, so that they never have inky margins, (2) the pilei remain campanulate or conical during the discharge of the spores, and never finally become flat-topped with recurved margins in the manner characteristic of small Coprini toward the end of the spore-discharge period. In association with the final campanulate or conical form of the pileus it is to be noted that, for *Psathyrella disseminata* at least, (3) the gills remain adnate and do not become free from the stipe. In the smaller Coprini, the opening out of the pileus in its later stages is always accompanied by a distinct separation of the gills from the stipe.

Granted that *Psathyrella disseminata* is not a Coprinus, one may still ask : is not this fungus closely related to the smaller Coprini ? It seems to me that the answer to this question must be in the affirmative. *Psathyrella disseminata* and the smaller Coprini, e.g. *Coprinus ephemerus*, etc., not only possess in common the seven field characters which have been enumerated above, but also a number of microscopic characters which concern the hymenium and the surface of the pileus. Both have : (1) a continuous system of large laterally-united paraphyses which form the ground-work of the hymenium in which the basidia are embedded, (2) a reduction of the generations of basidia to a very small number (in most Coprini this number is two but in *Coprinus micaceus* it is the same as in *Psathyrella disseminata*, namely, four), (3) polymorphism of the basidia so that each generation has its characteristic length with the result that there is differential protuberancy (in most Coprini the basidia are of two lengths only but *Coprinus micaceus* and *Psathyrella disseminata* agree in having basidia of four different lengths), (4) a close resemblance in the construction of the mosaic-work of which the hymenium is composed in respect to the crowding and relative distribution of the different kinds of basidia and the paraphyses, (5) oval or rounded cells which constitute the exterior firm layer of the pileus, some of which are produced from swollen bases into long, unicellular, cylindrical or somewhat conical hairs which under moist conditions excrete drops of slime at their tips (the

similarity of these hairs in *Psathyrella disseminata* and *Coprinus curtus*, etc., was demonstrated by Knoll[1]), and (6) the occurrence on top of the pileus of a certain number of loose spherical cells more or less aggregated into scales (there are but few of these loose cells in *Psathyrella disseminata* but they are quite similar in their nature to those which make up the tiny scales in *Coprinus curtus* and the more bulky mealy covering of *Coprinus niveus* and *C. stercorarius*). To these resemblances which concern the fruit-bodies we may add certain others which have to do with the mycelium. *Psathyrella disseminata* and certain of the Coprini, e.g. *Coprinus domesticus*, have (7) the same kind of habitat, namely, bark and wood, for they grow about dead roots and tree stumps. Moreover (8) these two species both produce an ozonium.

From what has been said in the foregoing paragraphs, I think that there can be no doubt that *Psathyrella disseminata* and many of the smaller Coprini have been evolved from a common stock and are nearly related. Without much difficulty, one can imagine a Coprinus arising from the Psathyrella or the Psathyrella from a small Coprinus. To change *Psathyrella disseminata* into a Coprinus it would only be necessary: (1) to make the gills parallel-sided, (2) to cause the pileus to open out more during the process of spore-discharge, (3) to alter the order of ripening and discharge of the spores so that these processes should proceed from below upwards on each gill instead of evenly all over the hymenium, and (4) to introduce the phenomenon of autodigestion of the gills from below upwards so that the spore-free portions of the gills should be systematically removed during the spore-discharge period. One could turn a small Coprinus into a Psathyrella by making an opposite set of changes. I know of no other fungus which could so readily be turned into a typical Coprinus as *Psathyrella disseminata*. My conclusion in regard to this fungus, therefore, is that, while it is not a Coprinus, it is very closely related to certain species of that genus. Possibly it belongs to a group of Melanosporae from which the Coprini have actually been evolved.

[1] F. Knoll, "Untersuchungen über den Bau und die Function der Cystiden und verwandter Organe," *Jahrb. für wiss. Bot.*, Bd. 50, 1912. Knoll's *Coprinus ephemerus* appears to be *C. curtus*.

CHAPTER III

THE BOLBITIUS SUB-TYPE ILLUSTRATED BY BOLBITIUS FLAVIDUS

Characters of the Bolbitius Sub-type—*Bolbitius flavidus*. General Description—The Spore-discharge Period—The Gills—The Hymenium—Correlation of Facts—*Bolbitius species B*—A Comparison of the Fruit-body Mechanism of Bolbitius and Coprinus

Characters of the Bolbitius Sub-type.—The Bolbitius Sub-type of fruit-body organisation possesses all the general characters already described for the Aequi-hymeniiferous Type : the gills are wedge-shaped in cross-section and positively geotropic, the hymenium looks downwards to the earth, and every part of the hymenium produces and liberates spores during the whole period of spore-discharge.

The Bolbitius Sub-type of organisation has been met with only in the genus Bolbitius. The fruit-bodies of all species of Bolbitius, as is well-known from field observations, are very ephemeral : they open, shed all their spores within about 24 hours, and then die down. Correlated with this ephemeral existence are : (1) the weak structure of each fruit-body as a whole, and (2) the special structure and mode of functioning of the hymenium. The fruit-bodies of the Bolbitius Sub-type, like those of the Psathyrella Sub-type, are very lightly constructed, the economy in building material being evidently related to the brevity of the spore-fall period and to the fact that the fruit-bodies are required to maintain themselves mechanically erect only during a very temporary existence. In their ephemeral nature and lightness of construction, the fruit-bodies of the Bolbitius Sub-type contrast strongly with the more stoutly built and persistent fruit-bodies which are included in the Panaeolus, the Inocybe, and the Armillaria Sub-types. The

chief difference in the fruit-body mechanism of the ephemeral fruit-bodies of the Bolbitius and Psathyrella Sub-types is to be found in the organisation of the hymenium.

We shall now consider in detail the special characters of the Bolbitius Sub-type. These are as follows :

(1) The whole structure of the fruit-body is relatively light and suited to a brief duration of the pileus.

(2) The spore-discharge period is very brief ; but, unfortunately, up to the present, I have not found an opportunity to measure it exactly. However, I have frequently noticed that fruit-bodies which expand one day die down the next. Moreover, the ephemeral nature of the fruit-bodies of the Bolbitii has been remarked upon by numerous authors, several of whom have likened the genus Bolbitius to Coprinus. We are therefore justified in assuming that the length of the spore-discharge period in the Bolbitius Sub-type is about 24 hours.

(3) The gills are not mottled, and there are no waves of development passing over the hymenial surface ; the hymenium develops everywhere in a very even manner. In this respect we have a distinct contrast with the Panaeolus Sub-type.

(4) As a rule, it is impossible to determine the number of generations of basidia which come up on a small area of the hymenium during the spore-discharge period, owing to the fact that, on such a small area, at any one time, one can observe basidia in all stages of development unassorted into groups of any kind. But sometimes it happens that, on a small area, the basidia and paraphyses take on a remarkably regular pattern and the basidia develop in successive groups. One can then observe that the number of generations of basidia which develop is four. This is a great reduction on the number of successive generations which are found in the Panaeolus Sub-type, but is equal to the number in the Psathyrella Sub-type.

(5) Adjacent basidia do not develop spores simultaneously. Basidia with spores of about the same age are a little distance apart. Younger basidia, which begin to develop spores between older ones bearing spores, are usually situated in the middle of the spaces between the older basidia, so that younger basidia do not interfere mechanically with older ones.

(6) The basidia are monomorphic, *i.e.* equally protuberant. In this respect the Bolbitius Sub-type agrees with the Panaeolus, the Inocybe, and the Armillaria Sub-types, but differs from the Psathyrella Sub-type, and from all the Coprinus Sub-types which make up the Inaequi-hymeniiferae. The amount of protuberancy of the basidia beyond the general level of the paraphyses is considerable, but not nearly so great as that of the longest set of basidia in *Psathyrella disseminata*, *Lepiota cepaestipes*, and the Coprini.

(7) There is no lateral crowding of the basidia with overlap of spores such as is found in the Psathyrella Sub-type and in the Coprinus Sub-types, but adjacent spore-bearing basidia are separated by spaces equivalent to those found in the Panaeolus Sub-type.

(8) The paraphyses are relatively large and well-developed. They are joined together laterally so as to form a thin flat sheet of tissue through which the isolated basidia protrude. A similar system of paraphyses occurs in the ephemeral Psathyrella and Coprinus Sub-types, but is absent from the Panaeolus, the Inocybe, and the Armillaria Sub-types which have more persistent fruit-bodies. In the latter Sub-types, the paraphyses are relatively smaller and not linked into a continuous system. The paraphyses act as space-making agents in that they separate the basidia during the production and liberation of the spores; and, by their rapid growth, they also serve to enlarge the superficial area of the gills during the rapid expansion of the pileus.

(9) In the two species of Bolbitius which I have examined, the basidia have a peculiar shape which may well be characteristic for the whole genus. The inner half of each basidium consists of a narrow cylindrical or slightly tapering shaft entirely enclosed by paraphyses. This shaft, on passing out between the paraphyses to the exterior of the hymenium, suddenly bulges into a more or less globular swelling which constitutes the outer half of the basidium. This swelling is the protuberant part of the basidium and upon it arise the sterigmata.

From the above, it will be noticed that the chief difference between the Bolbitius and the Psathyrella Sub-types lies in the fact that, whereas in the former the basidia are monomorphic, not crowded laterally, and not as a rule arranged in a definite series of

successive generations, in the latter the basidia are polymorphic, crowded laterally so that there is overlapping of the spores, and arranged in a definite series of generations which usually are four in number.

Bolbitius flavidus. General Description. — The fruit-bodies which were used for my observations were found coming up on wet matted dead grass in a pasture at Birmingham, England (Fig. 30). Horses had been feeding in the pasture from time to time, but an examination of the dead grass seemed to show that the mycelium had vegetated, not upon horse dung, but solely upon the dead grass. The fungus came up in the same pasture for several years in succession, and was observed in the months of June, July, and August. The fruit-bodies resembled in appearance those shown in Plate 689 in Cooke's *Illustrations of British Fungi*. The species there represented, according to Massee,[1] is *Bolbitius flavidus* Bolt. I shall therefore use this name for my species. However, I feel some doubt in regard to the identification, for Bolton's original description fits neither Cooke's illustration nor my specimens in regard to stature. Bolton says that the stipe is 2 inches high ; but Cooke's illustration shows them to be from $2\frac{1}{2}$ to $3\frac{1}{2}$ inches high, while my specimens were mostly about 3 inches to $3\frac{1}{2}$ inches high, with an occasional specimen as much as 5 inches high. The following is a general description of the species, made from my own specimens.

The pileus, before expansion, is at first ovate and then obtusely conical, slightly plicate at the base, about 1·5 cm. high but occasionally higher, decidedly yellow especially at the apex, and covered by a continuous, separable, viscid pellicle. On opening out, the pileus becomes campanulate and finally flattened with the disc slightly depressed (Fig. 30, E). During the expansion of the pileus, the gills split radially from above downwards along their median planes, as in *Coprinus plicatilis*, *Psathyrella disseminata*, etc., so that the outer part of the pileus becomes striate and sulcate. The expanded pileus is usually from 2·5 to 3·5 cm. in diameter but exceptionally as much as 8 cm., not infrequently torn radially half way to the centre in one or two places, bright yellow at the centre passing into dull chocolate-yellow toward the periphery, and

[1] G. Massee, *British Fungus-Flora*, vol. ii, p. 204.

becoming paler with age. The viscid pellicle, during the expansion of the pileus, fails to keep pace in its growth with the pileus-periphery so that, when the pileus has become flattened, it is found to be restricted to the central region of the pileus including the disc. Its retirement from the peripheral zone of the pileus leaves the

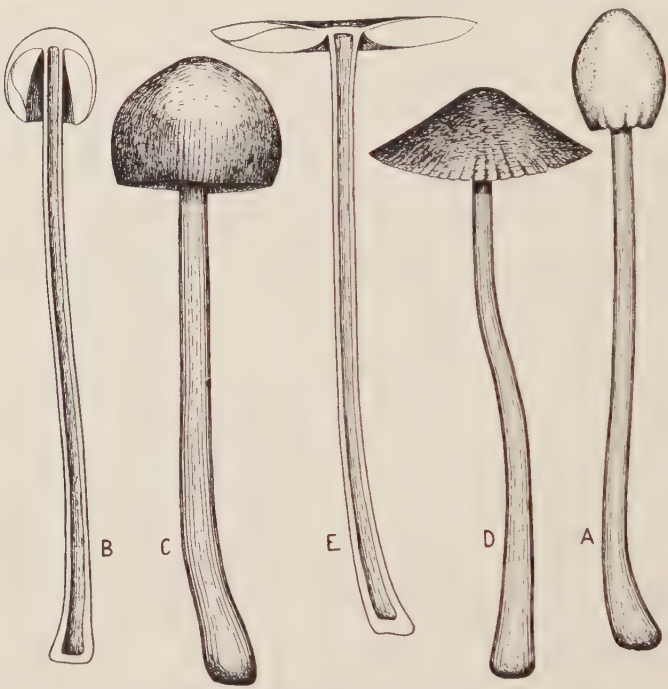


Fig. 30.—*Bolbitius flavidus.* Fruit-bodies coming up in a grassy field near Birmingham, England. A, unexpanded; B, C, and D, expanding; E, fully expanded and shedding spores without autodigestion of the gills. Natural size.

delicate gills in that region without a covering. The dull chocolate-yellow colour of the peripheral portion of the pileus is in part due to the somewhat chocolate-coloured spores, which show through the translucent gills.

The gills are always extremely thin and watery; in pilei having a diameter of 3·5 cm. they are 1·5 cm. long and 0·4 cm. broad at the centre, but in an exceptionally large pileus having a diameter of 8 cm. they may be 3·5 cm. long and 0·7 cm. broad. At first, in the unexpanded pileus, they are yellowish-white; but, as the pileus

opens out, they become dull chocolate-yellow. Eventually some of them often become powdered on one side with the spores.

The stipe is usually from 6 to 9 cm. high when fully elongated, but occasionally it attains a height of 12 to 13 cm. Its base is somewhat swollen and 0·5 to 0·6 cm. in diameter. The main shaft is cylindrical or very slightly tapering upwards. At its centre it is usually 0·3 to 0·4 cm. thick but, in very large fruit-bodies, it may be 0·5 to 0·6 cm. thick. Its general colour is white tinged with yellow. Below, its surface is somewhat roughened with whitish fibrils, but above, just beneath the pileus, it is smoother and exhibits longitudinal striæ. Within, it is hollow. As a whole, it is very fragile like the stipes of many Coprini.

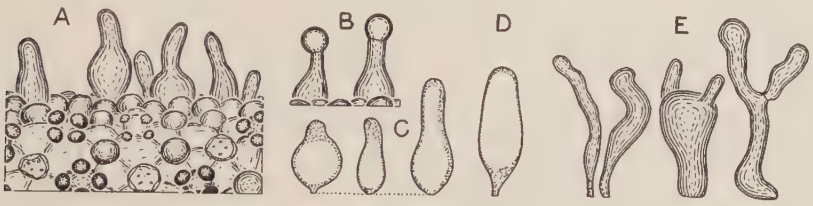


Fig. 31.—Cystidia of *Bolbitius flavidus*. A, cheilocystidia at the free edge of a gill. B, two cheilocystidia with a drop of excreted fluid at each apex. C, three cheilocystidia showing protoplasm and vacuoles. D, a pleurocystidium from the side of a gill, showing shape and contents. E, caulocystidia from near the top of the stipe. Magnification, 293.

There are no pilocystidia on the top of the pileus, but the other three types of cystidia are all represented. (1) The cheilocystidia, disposed along the free edge of each gill (Fig. 31, A), are ventricose below and extended above into a short cylindrical neck. They contain a large vacuole (C), and sometimes one finds a drop of fluid excreted at their apex (B). Size : 30–50 μ long, 12–20 μ wide. (2) Pleurocystidia, scattered on the sides of the gills, are but rarely seen (D) and in some specimens possibly may be entirely absent. They are ovate-cylindrical, contracted into a short stalk at the base, and bluntly rounded at the apex ; and each contains a large vacuole. Size : about 60 μ long and 20 μ wide. (3) The caulocystidia, toward the top of the stipe, occur in tufts and consist of simple or branched hyphae of somewhat variable shape (E). Size : 50–80 μ long, 10–30 μ wide.

The spores are oval, 11–12 μ long and 7 μ broad, bright yellow

when seen under the microscope but rusty-brown in deposits formed on the stipe or on white paper.

The fruit-bodies are found either singly or a few together growing on dead matted grass in pastures in England during June, July, and August. They are very fragile and, after shedding their spores, soon become melted down into a brown fluid.

The Spore-discharge Period.—The length of the spore-discharge period for *Bolbitius flavidus*, unfortunately, has not yet been exactly measured, owing to difficulties in obtaining fresh fruit-bodies when it was possible to make the necessary observations. However, there can be no doubt that it is a very short one. A fruit-body was gathered from a field at noon, when the pileus was still in the campanulate condition, just as expansion had begun and before any spores had been liberated, and the base of its stipe was placed in water in a cup. Next morning the pileus was found to have become fully expanded and to have already produced a thick spore-deposit. Later on in the day, at some undetermined time, spore-discharge ceased ; and, by the morning of the third day, the pileus was found to have melted down into a brown and glutinous fluid. These observations, together with others made upon fruit-bodies growing wild, have convinced me that the spore-discharge period is not longer than 24 hours. There can be no doubt, therefore, that the expanded fruit-bodies of the species under discussion have but a very ephemeral existence. With this ephemeral existence is correlated the weak and watery structure of the fruit-body as a whole and the peculiar organisation of the hymenium.

The Gills.—The gills of *Bolbitius flavidus* are not parallel-sided but acutely wedge-shaped ; and they are also positively geotropic. The geotropism was proved by direct experiment. A young fruit-body was placed in such a position that, during expansion, the top of the pileus, instead of becoming stretched out horizontally, became tilted at an angle of 30° to a horizontal plane. The gills adjusted themselves about their lines of attachment to the pileus-flesh so that they all came to direct themselves exactly downwards toward the earth ; and, in making this adjustment, some of them turned through an angle of 30°. In a normally oriented fruit-body, such as one sees in a field, on account of the shape and geotropic

reaction of the gills, every part of the hymenium comes to look downwards, as is shown in the cross-section represented in Fig. 32. Further, every part of the hymenium (every square mm.) produces and liberates spores at one and the same time during the whole period of spore-discharge. This aequi-hymeniiferous character is indicated in Fig. 32 by the arrows which show the trajectories of a number of spores which, conceivably, have been shot away from various parts of the hymenium within a single minute. It is therefore clear that the spores on each gill do not ripen and become discharged from below upwards; nor, during the spore-discharge period, does any autodigestion destroy the gills from below upwards. In all these respects, therefore, the fruit-bodies of *Bolbitius flavidus* resemble those of the Panaeolus and the Psathyrella Sub-types, and differ from those of the Coprinus Sub-types which make up the Inaequi-hymeniiferae.

FIG. 32.—*Bolbitius flavidus*. Vertical section through pileus showing the thin flesh and wedge-shaped gill-sections. The arrows show the trajectories of the spores which are being shed from all parts of the hymenium. Magnification, 30.

The gills are extremely thin. Near the pileus-flesh, the long ones only just exceed 0·2 mm. in thickness (Fig. 32), whilst near the free gill-edges they are only 0·1 mm. thick. In the Coprini alone do we meet with thinner gills than these. Now the gills, although wedge-shaped in cross-section and very sensitive to the stimulus of gravity,

do not always succeed in obtaining a suitable orientation for securing the discharge of all their spores. One often notices that one side of a gill is powdered with fallen spores whilst the other is not. The powdering is due to the misplacement of the gill : the powdered side has looked slightly upwards, and has therefore caught a large number of spores which have fallen vertically after being shot away from the hymenium. Such misplacement of a gill may be brought about by various causes, such as the displacement of the pileus owing to the action of the wind on the fruit-body as a whole, the partial drying of the free edges of the very delicate gills, etc. If the gills were not so acutely wedge-shaped and weakly constructed, doubtless powdering would occur less often. It is interesting to note that similar powdering occurs upon the gills of *Coprinus plicatilis*, which in general form resembles *Bolbitius flavidus*.

The Hymenium.—In face view, the hymenium exhibits not a trace of the mottling which is so characteristic of the Panaeolus Sub-type ; but instead, on every small area of the gill-surface, basidia are to be found bearing spores in various stages of development. The appearance of the spores on one small area is shown in Fig. 33 at B. This drawing, which may be contrasted with similar drawings for *Panaeolus campanulatus* given in Volume II (pp. 257, 258), shows not only the absence of mottling but also that basidia which bear spores of about the same age are well separated from one another. All the elements of the hymenium, including the spores of B, are shown at A which represents a stage of hymenial development just previous to the beginning of spore-discharge. Here, it will be observed, the basidia appear to be developing in no very regular manner. One cannot speak of a definite first, second, or third generation of basidia, etc., for the basidial population shows all gradations of age on one small area. However, one rule for spore-production is generally kept, and that is that adjacent basidia shall not produce spores at one and the same time. This ensures mutual mechanical non-interference between the spores of neighbouring basidia.

The elements of the hymenium, as a rule, are two only—basidia and paraphyses. Cheilocystidia occur along the free edge of each gill, but only very rarely have I seen an occasional pleurocystidium

placed well on the side of a gill among the basidia. The paraphyses are large sterile elements resembling those of *Psathyrella disseminata*,

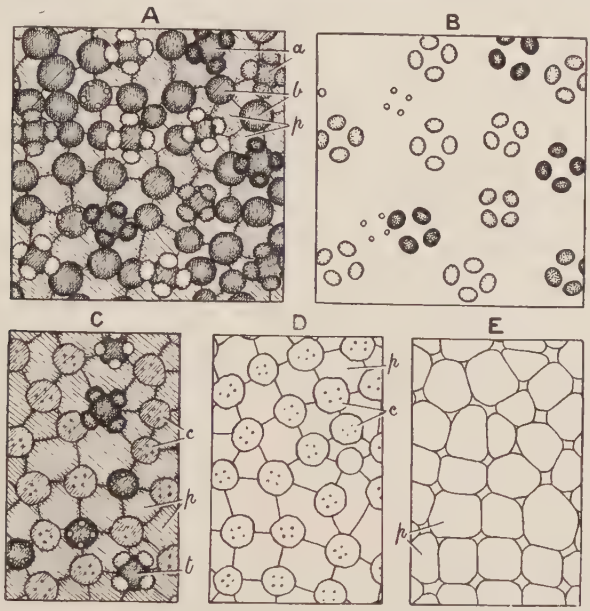


FIG. 33.—*Bolbitius flavidus.* Analysis of the hymenium as seen in surface view. A, an area one-tenth of a mm. square in extent, just before the beginning of the spore-discharge period. Basidia of various ages, but no two of like age, are close together; *a*, older basidia bearing spores; *b*, younger basidia which have not yet developed spores; *p*, paraphyses. Fourteen of the basidia bear spores and thirty-one are still without any. B, the spores of the fourteen spore-bearing basidia of A set out separately; the degree of pigmentation of the spores is shown by the amount of shading. C, a piece of hymenium near the end of the spore-discharge period. There are now only six turgid basidia, *t*, all producing spores but of different ages; the other basidia, *c*, have collapsed; *p*, paraphyses. D, a piece of hymenium from an exhausted gill. All the basidia, *c*, have now collapsed; *p*, paraphyses. E, a piece of hymenium in which only the paraphyses, *p*, have been sketched. Through the spaces between them were inserted the stalks of the basidia. Magnification, 352.

Lepiota cepaestipes, and Coprinus. They are welded together into a single system which makes a ground-work through which the narrow shafts of the isolated basidia protrude (Fig. 34, D and E). They constitute the elastic elements of the hymenium and serve to

create spaces between adjacent basidia. When the hymenium is very young, before spore-discharge has begun and whilst the gills are still inclined downwards in a direction more or less parallel to the axis of the stipe, the basidia are packed very closely together, as is shown in Fig. 34 at A; but, as the pileus expands and the gills become stretched more and more horizontally, the basidia come

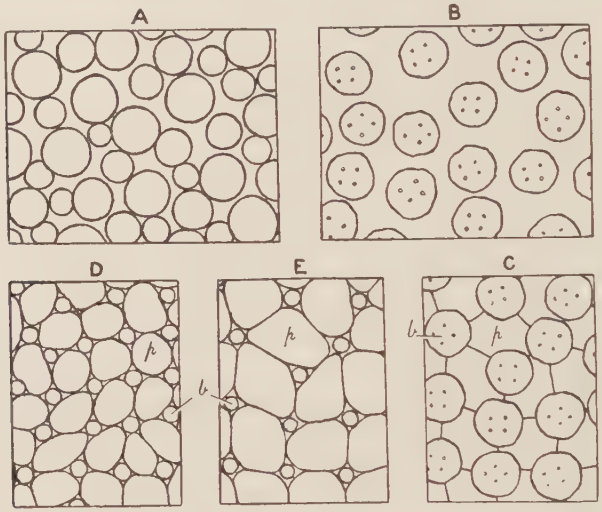


FIG. 34.—*Bolbitius flavidus*. Comparison of a very young hymenium with a very old hymenium, in surface view, to show the effect of the swelling of the paraphyses on the increase of area of the gills. A, very young hymenium before the production of spores; B, very old hymenium after the discharge of the spores; only the basidia have been sketched. D, very young hymenium; E, very old hymenium; both showing paraphyses, *p*, and the stalks of the basidia, *b*. C, the same as E, but showing not only the paraphyses, *p*, but also the tops of the collapsed basidia, *b*. Magnification, 500.

to be more freely spaced, as shown in Fig. 34 at B. The hymenial growth during the stretching and increase in size of the gills, just as in the Coprini, is brought about entirely by the increase in size of the paraphyses. The growth of these cells may be realised by comparing the drawings D and E in Fig. 34. D represents a surface-section through a very young hymenium, the larger elements being the paraphyses and the smaller the narrow shafts of the basidia. E shows a similar drawing for a fully expanded hymenium. The

comparison makes it clear that during the expansion of the pileus the paraphyses about double their superficial area, whilst the shafts of the basidia remain unchanged. The number of paraphyses which enclose the shaft of each basidium varies from three to five, but is most often four.

The basidia are monomorphic, *i.e.* they are all of about the same length and equally protuberant at maturity. This is shown in Fig. 35 which represents semi-diagrammatically a cross-section through the hymenium and subjacent cells. The monomorphism of

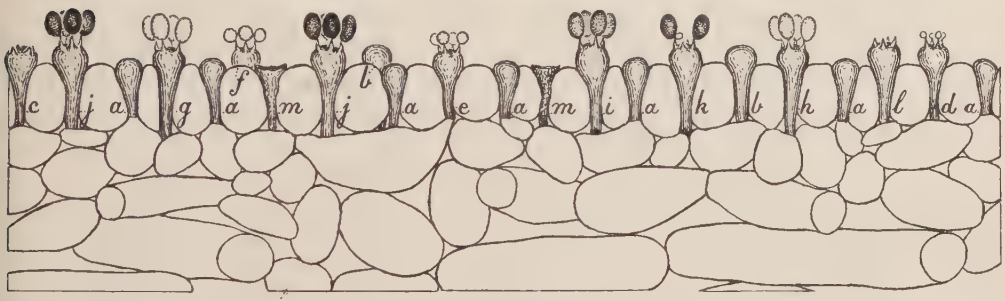


FIG. 35.—*Bolbitius flavidus*. Semi-diagrammatic vertical section through the hymenium shortly after the beginning of spore-discharge. The paraphyses and subhymenium are unshaded. The basidia are in various stages of development: *a*, youngest basidium, not projecting; *b*, older basidium, projecting above paraphyses; *c*, sterigmata developing; *d*, spores developing; *e* and *f*, spores growing larger; *g*, spores full size but unpigmented; *h*, *i*, *j*, spores becoming brown; *k*, spore-discharge in progress, two spores have already been discharged and a third, with a drop of water excreted at its hilum, is about to be discharged; *l*, all four spores have been discharged; *m*, collapsed basidium. Magnification, 352.

the basidia is correlated here, as in the Panaeolus Sub-type, with the fact that adjacent basidia do not bear full-sized spores at one and the same time. In being monomorphic and not polymorphic, the basidia of *Bolbitius flavidus* differ in a striking manner from those of *Lepiota cepaestipes*, *Psathyrella disseminata*, and all species of Coprinus, for in these fungi the basidia are dimorphic, trimorphic or tetramorphic.

The shape of the basidia is peculiar: the narrow shaft, on passing to the exterior of the hymenium, becomes rather suddenly swollen into a rounded extremity upon the end of which are developed the four sterigmata (Fig. 35). This form of basidium-body may be of general occurrence in Bolbitius, for I have noticed it in another

species of that genus. The protuberancy of the basidia of *Bolbitius flavidus*, while marked, is not nearly so great as that of the longest basidia of *Lepiota cepaestipes*, *Psathyrella disseminata*, and the Coprini. It is no greater than that characteristic of the fruit-bodies of the Panaeolus Sub-type ; and, as in *Panaeolus campanulatus*, it just suffices to secure that each basidium shall get its top well above the external walls of the paraphyses and above any wasted spores that may cling to them, the result being that the sterigmata and spores freely develop without mechanical hindrance.

Various stages in the development of the basidium are to be seen represented in Fig. 35. The youngest basidium, *a*, is non-protuberant ; it next becomes fully protuberant, *b* ; it then develops sterigmata, *c* ; then spores, *d*, *e*, *f*, *g* ; which gradually turn brown, *h*, *i*, *j* ; and are then discharged, *k* ; leaving bare sterigmata, *l*. The basidium then collapses and its end becomes concave, *m*, so that the remains of the sterigmata, which soon dwindle to mere stumps, disappear from sight in the usual manner. A few seconds before the discharge of each spore a drop of fluid is excreted from the spore-hilum, as in *Panaeolus campanulatus*, etc. This is shown at *k*. Other general points shown in Fig. 35 are : (1) the isolation of the basidia from one another by the broad paraphyses, and (2) the fact that basidia with spores of about the same age are not adjacent to one another but are situated relatively far apart.

It has already been shown that, as a rule, at any given time, on any one small area of the hymenium, the basidia are in all stages of development so that one cannot divide them into a series of successive generations. This rule, however, is not invariably kept, for it sometimes happens that the basidia and paraphyses in a very limited hymenial area take on a remarkably regular pattern. One then perceives that the paraphyses are four-sided and arranged like the squares on a chess-board, and that there is a basidium situated at every point where four paraphyses meet. Moreover, the basidia are divided into four distinct sets which are equal in number and equally spaced, and which bring their spores to maturity in succession so as to form four equal and successive generations. The whole arrangement is shown diagrammatically in Fig. 36, A, where the squares represent the paraphyses and the circles the basidia.

The black circles represent the positions of the basidia of the first generation to produce spores, the cross-hatched circles the positions of the second-generation basidia, the circles shaded with lines the positions of the third-generation basidia, and the unshaded circles the positions of the fourth-generation basidia. The actual arrangement of the basidia in one small area of the hymenium is shown in Fig. 36, B, where the tendency to form the pattern given in the diagram can undoubtedly be recognised. The drawing was made

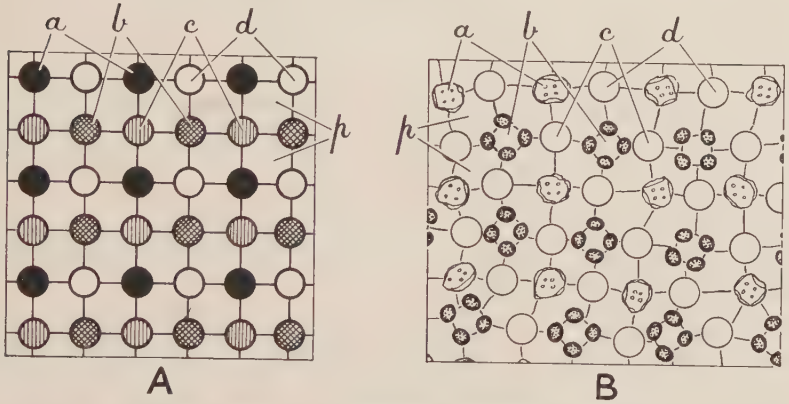


FIG. 36.—*Bolbitius flavidus*. A, a diagram showing a regular arrangement of the elements of the hymenium, the basidia appearing as circles and the paraphyses, *p*, as squares. There are four successive generations of basidia, *a*, *b*, *c* and *d*, each generation represented by nine basidia. The basidia *a* (black) belong to the first generation, *b* (cross-hatched) to the second generation, *c* (shaded with lines) to the third generation, and *d* (plain) to the fourth generation. B, a *camera-lucida* drawing of an actual hymenium showing an almost regular arrangement corresponding to that of A. The first-generation basidia, *a*, have shed their spores. The second-generation basidia, *b*, are now bearing spores. The third- and fourth-generation basidia, *c* and *d*, cannot at present be distinguished from one another. *p*, the paraphyses. Magnification, 345.

at the time when the second generation of basidia was ripening its spores, and this generation could easily be distinguished by their presence. It is noteworthy that even the groups of four spores exhibit a distinct tendency to orient themselves in one definite manner, and not according to the laws of chance. The first-generation basidia, *a*, are represented by the collapsed basidia which have already shed their spores and which are about equal in number to those of the second generation, *b*. The third-generation and fourth-generation basidia, *c* and *d*, are represented by young basidia

which have not as yet produced either sterigmata or spores. I was not able to distinguish the third-generation basidia from those of the fourth generation, but simply assume that their numbers and arrangement are like those of the basidia of the first and second generations. A study of Fig. 36, B, will show that irregularities creep into the pattern here and there, and that there is no such mathematical exactitude displayed as in the diagram. This, however, is what one might expect in a living organism, and does not weaken the general conclusion that the elements of the hymenium tend to arrange themselves locally in a symmetrical manner. It was stated that, in ordinary areas of the hymenium where there is no such regular arrangement of the elements as has just been described, the basidia which are beginning to develop spores are as far away as possible from the basidia which already bear spores. The same tendency can be seen where we have the regular pattern, as will appear from a study of the diagram A in Fig. 36. Let us note the position of the basidia of the first generation, *a*. The basidia of the second generation, *b*, arise in the middle of the squares formed by these first-generation basidia and not in the other possible locations, *i.e.* they arise in the positions which are the most remote from the basidia of the previous generation.

An attempt was made, by means of the method [1] described in Volume II. to determine the length of time occupied in the development of the spores of individual basidia. A gill was dissected from a newly gathered fruit-body and laid flat in a compressor cell. A drop of water was then added and the cell closed by its cover. The hymenium was observed in face view with the low power of the microscope. Unfortunately, the basidia continued to grow normally for only a short time, and then the spores on each collapsed together. Several trials with varying amounts of water in the cell convinced me that the isolated gills are very sensitive to changes in the amount of water or water-vapour with which they are in contact, and do not long continue normal development in a small compressor cell. For a single gill a large piece of pileus, including several gills, was substituted. This piece of pileus was inverted from its normal position so that the gills looked upwards. Develop-

[1] These *Researches*, vol. ii, 1922, pp. 43–48.

ment of the spores on several basidia was watched for some hours: the spores were seen to begin their development as minute rudiments on the ends of the sterigmata, grow to full size, remain colourless for somewhat over an hour, and then become more and more deeply coloured with a golden-yellow pigment; but the sterigmata ultimately collapsed and dragged the spores toward the hymenium without discharging them. Complete normal development and discharge, therefore, was not observed for any single basidium. However, it is my opinion, based on these observations and numerous observations of a more successful character with other species, that several hours are required for a basidium of *Bolbitius flavidus* to develop and ripen its spores up to the moment of discharge. I am inclined to believe that these hours number at least six and may be eight. If we assume that seven is the right number, then we can calculate the length of the spore-discharge period for those parts of the hymenium where four generations of basidia can be made out definitely. After the first generation of basidia has shed its spores, three other successive generations will take about twenty-one hours to produce and shed their spores, *i.e.* the spore-discharge period will be a little less than twenty-four hours in length.

Correlation of Facts.—Let us now attempt to correlate the various facts connected with the organisation of the fruit-bodies. The fruit-bodies of *Bolbitius flavidus* shed all their spores within twenty-four hours and then immediately collapse; and they usually come up after rains when the air is moist and relatively still. Since they have such an ephemeral existence under these conditions, it is not necessary for them to be stoutly built like Polypori, Boleti, *Psalliota campestris*, *Lepiota procera*, etc., all of which continue to shed spores for many days even during very dry weather. Accordingly, we find that they are weak and watery: for the maintenance of their rigidity they depend chiefly on the turgor of their cells. The stipe is a hollow cylinder with a wide cavity and relatively thin walls, and resembles the stipes of such equally ephemeral fruit-bodies as *Coprinus niveus* and *C. macrorhizus*: the minimum amount of material is so disposed as to obtain the maximum mechanical effect for resisting the strains to which

the stipe is likely to be subjected. The flesh of the pileus is also very thin and, in the saving of substance thus effected, we are reminded once more of other ephemeral fruit-bodies, namely, those of Coprini, *Psathyrella disseminata*, and *Lepiota cepaestipes*. The splitting of the gills from above downwards in the outer part of the pileus takes place just as in *Coprinus lagopus*, *Psathyrella disseminata*, etc., and is a rapid and very simple means of opening out the pileus without any corresponding growth of the pileus-flesh. The gelatinous pellicle, which covers the entire surface of the pileus before the fruit-body expands, fails to extend as the pileus opens, with the result that the outer part of the pileus, which in the extended condition becomes exceedingly thin and delicate, is unable to resist for long any drying breeze to which it may be exposed. The gills are not stiff and tough, but are very watery. The hymenium is so organised that all the basidia can shed their spores in the short period of time during which the pileus is expanded. The basidia, relatively to the paraphyses, occupy a smaller proportion of the hymenium than they do in *Psalliota campestris* and other members of the Panaeolus Sub-type, where the spore-discharge period is a long one. The number of basidia to come to maturity on any unit area of the hymenium is therefore reduced in correlation with the ephemeral existence of the pileus. Where the elements of the hymenium are arranged in a very regular pattern, as sometimes happens, it can be observed that the number of generations of basidia is four only—a number corresponding to that of the ephemeral fruit-bodies described in connection with the Psathyrella Sub-type, but contrasting with that of the persistent Panaeolus Sub-type where it is at least twice as great. The paraphyses are relatively very large and are united into a system which forms a ground-work or pavement containing and isolating the basidia, just as in the Coprini, *Psathyrella disseminata*, and *Lepiota cepaestipes*. No doubt they serve to give mechanical strength to the very watery gills as in the other fungi mentioned and, at the same time, by their rapid increase in size, aid in the process of rapid pileus-expansion when this takes place. A continuous system of large paraphyses, such as we are discussing, contrasts with the irregular strings of relatively small paraphyses such as we find in

the Panaeolus Sub-type, and appears to be associated only with ephemeral fruit-bodies which open rapidly.

Bolbitius species B.—Another species of Bolbitius was found

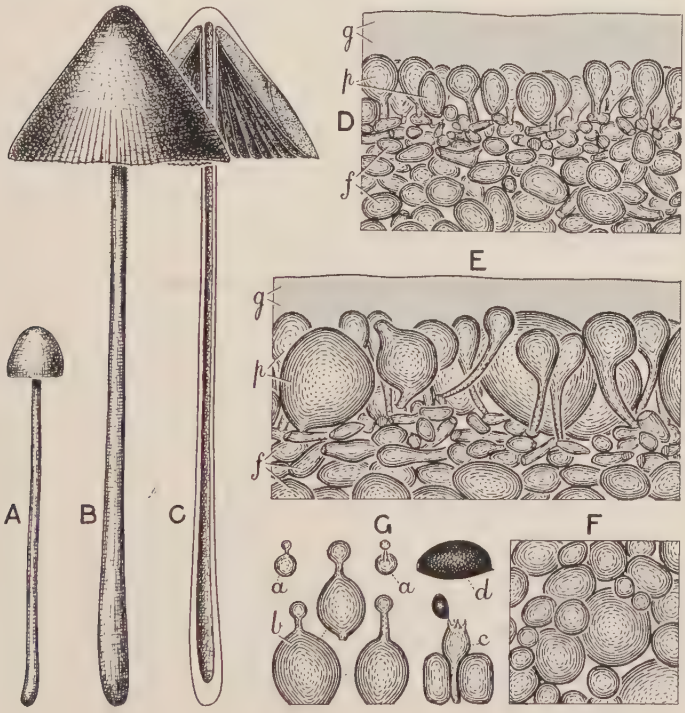


FIG. 37.—*Bolbitius species B.* A, a fruit-body of small size, not yet fully expanded. B, an older fully expanded fruit body. C, a vertical section through B. D and E, vertical sections through the top of the pileus of A and B respectively: *g*, a transparent gelatinous membrane formed from the outer walls of the palisade cells *p*; *f*, other cells of the pileus-flesh. F, a surface view of E showing the tops of palisade cells seen through the transparent gelatinous membrane. G: *a a*, two cheilocystidia from the free edge of a gill; *b*, three cheilocystidia much magnified; *c*, a basidium and two paraphyses; *d*, a spore much enlarged. A, B, C, natural size. Magnification: D, E, F, G *a* and *c*, 293; G *b* and *d*, 746.

coming up on the dunged soil of a Cucumber-bed in a green-house at Birmingham, England (Fig. 37, A, B, C). Its pileus was at first conical, brownish-buff on the flanks, a little darker at the apex, and about 2 cm. high. The gills were 20 mm. long, 2 mm. wide, and light chocolate-brown in colour. The stipe was 9·5 cm. high,

cylindrical above, about 2·5 mm. thick in the centre, slightly thicker below, hollow, white, smooth below and slightly fibrillose just below the pileus. The spores were rich yellow when seen in water with the microscope, and formed a chocolate-coloured deposit on white paper. Their dimensions were 13–14 by 7·5 μ and in shape they were oval-fusiform (Fig. 37, G, *d*). The above description was made from the larger of the two fruit-bodies. The smaller fruit-body was only about one-half the size of the larger. I was unable to identify the species, but two mycological friends have suggested that it was *Bolbitius titubans*; but, as I feel uncertain about this identification, I prefer to call it *Bolbitius species B*.

The general structure of the hymenium of *Bolbitius species B* was found to be identical with that of *Bolbitius flavidus*. The paraphyses were again relatively large and united into a definite system, while the basidia were monomorphic and swollen at their ends just as before (Fig. 37, G, *c*). The organisation of the whole fruit-body was so similar to that of the Bolbitius which has just been described that it will not be necessary to give an account of it in detail. It seems to me highly probable, therefore, that all the other species of Bolbitius will be found to be organised like *Bolbitius flavidus* and thus come to be included in the Bolbitius Sub-type.

At D and E in Fig. 37 are shown two vertical sections of the pileus of *Bolbitius species B*, D having been made from the specimen A, and E from the specimen B. In both, at *g*, there is a well-marked transparent gelatinous pellicle. This pellicle, which resembles that of *B. flavidus*, appears to have been produced by the swelling of the outer walls of the palisade layer of the pileus-flesh.

The cheilocystidia of *Bolbitius species B* differ from those of *B. flavidus*, and at the same time closely resemble those of *Galera tenera*, in that their necks are very thin instead of being stout and in that each neck, instead of being naked, is crowned apically by a rounded swelling or head-piece (Fig. 37, G).

A Comparison of the Fruit-body Mechanism of Bolbitius and Coprinus.—A comparison of the fruit-body mechanism of Bolbitius and Coprinus was made by the writer[1] in 1913 with a view

[1] A. H. R. Buller, "The Fruit-body Mechanism of Bolbitius," *Transactions of the British Mycological Society*, 1913, pp. 235–238.

to throwing light upon the systematic relationship of the two genera ; and it is repeated here in its original form with but slight additions.

In treating of the Hymenomycetes several writers have referred to the resemblances which exist between the genera Bolbitius and Coprinus. Thus Massee [1] says : " In the Ochrosporae, the genus Bolbitius agrees with Coprinus in the ephemeral existence of the species, in the soft deliquescent gills, and also in most frequently growing on dung or in places where dung abounds." Referring to Bolbitius the same writer [2] also says : " Very delicate and fragile, remarkable amongst the Ochrosporae for the gills dissolving into mucus, and in this respect analogous with Coprinus among the Melanosporae and Hiatula among the Leucosporae."

The genus Bolbitius was included by Hennings [3] along with Coprinus in the group Coprineae, which was defined as follows : " Fruit-bodies with soft flesh (weich-fleischig), composed of an even web of hyphae, stipitate. Lamellae of different lengths, alternating in a regular manner. Hymenium composed of isolated protuberant basidia which are separated from one another in a regular manner by paraphyses. Lamellae, and usually also the cap, deliquescent."

In previous publications, I [4] have described the fruit-body mechanism of the Coprini and have shown that the so-called deliquescence is a process of autodigestion which plays an essential part in the liberation of the spores.[5]

In the genus Coprinus :

(1) The gills are very thin.

(2) The gills are parallel-sided or subparallel-sided.

(3) The gills are not positively geotropic.

[1] G. Massee, *British Fungus-Flora*, vol. i, 1892, p. 304.

[2] *Ibid.*, vol. ii, 1893, p. 203.

[3] P. Hennings, in Engler and Prantl's *Pflanzenfamilien*, Teil I, Abteil. I**, 1897, p. 204. The translation here given is mine.

[4] A. H. R. Buller, *Researches on Fungi*, vol. i, chap. xix ; also *Annals of Botany*, vol. xxiv, 1910, pp. 613–629 ; *Trans. British Mycological Society*, 1911, pp. 348–350 ; " Die Erzeugung und Befreiung der Sporen bei *Coprinus sterquilinus*," *Jahrb. f. wiss. Bot.*, Bd. 56 (Pfeffer-Festschrift), 1915, pp. 299–329.

[5] Also *vide infra* in the Chapters treating of the Coprinus Sub-types, particularly Chapter VIII.

(4) Usually the hymenium on one side of a gill at maturity looks slightly downwards, and that on the other side slightly upwards.

(5) The spores ripen in succession from below upwards on each gill.

(6) The spores are discharged in succession from below upwards on each gill.

(7) Autodigestion proceeds from below upwards on each gill and removes those parts of the gills which have become spore-free and which, if they continued in existence, would become mechanical hindrances to the fall of the remaining spores.

All the facts just given are correlated with one another. In the genus Coprinus the ripening and discharge of the spores from below upwards on each gill, and the autodigestion from below upwards on each gill, are special arrangements which permit of successful spore-discharge from parallel-sided, non-geotropic gills.

After the fruit-body mechanism of Coprinus had been elucidated, it seemed to me advisable to investigate the fruit-bodies of a Bolbitius with a view to finding out whether or not their arrangements for producing and liberating spores were the same as in Coprinus. I therefore examined the living fruit-bodies of *Bolbitius flavidus* Bolt. with the results already recorded in this Chapter.

In the fruit-bodies of the Bolbitius :

(1) The gills are very thin.

(2) The gills are not parallel-sided but are acutely wedge-shaped in cross-section.

(3) The gills are positively geotropic.

(4) Every part of the hymenium on any gill in a normally oriented fruit-body looks more or less downwards.

(5) The spores do not ripen in succession from below upwards on each gill.

(6) The spores are not discharged in succession from below upwards on each gill.

(7) Autodigestion does not proceed from below upwards on each gill ; it does not have any relation to spore-discharge, but is a *post-mortem* change.

It is clear that the special mode of spore-production and spore-

discharge which we find in Coprinus does not exist in Bolbitius. In particular, the liquefaction of the gills, which is such an essential feature of the fruit-body mechanism of Coprinus, has nothing to do with spore-discharge in Bolbitius. In Coprinus the autodigestion takes place whilst the pileus is still actively shedding spores, whereas in Bolbitius the turning of the pileus into a brown mucus takes place when spore-discharge has ceased and the whole pileus is exhausted and dead.

The fruit-body mechanism of Bolbitius resembles in its main features that of a Mushroom. The gills are wedge-shaped in cross-section and are positively geotropic. This ensures that in a normally oriented fruit-body every part of the hymenium looks more or less downwards. Correlated with this fact we find that every small area of the hymenium (every square mm.) produces and liberates spores at one and the same time. On any one small area a series of basidia ripen and shed their spores in succession.

From the foregoing remarks we may conclude that the arrangements for spore-production and spore-liberation in Bolbitius are essentially different from those of Coprinus.

The hymenium on the sides of the gills of Coprinus consists of :

(1) Basidia which are dimorphic : long basidia and short are interspersed among one another. In a very few species the basidia are either trimorphic or tetramorphic.

(2) Large and well-developed paraphyses which form a continuous system and separate the basidia from one another.

(3) Large pleurocystidia, present in some species but absent in others.

The hymenium on the sides of the gills of the Bolbitius consists of :

(1) Basidia which are monomorphic : the basidia are all of the same length and cannot be divided into two sets, long and short.

(2) Large and well-developed paraphyses which form a continuous system and separate the basidia from one another.

(3) Pleurocystidia are absent or very rare.

Coprinus and Bolbitius have very similar paraphyses which are remarkable for their high development. On the other hand the

two genera differ markedly in their basidia. The dimorphism which we find in Coprinus is absent in Bolbitius. Further, on any very small area of the hymenium of Coprinus at maturity all the basidia have full-sized and practically ripe spores on them at one and the same time. On a similar area of the hymenium of Bolbitius adjacent basidia are *not* in about the same state of development at any one time. Here the basidia develop and discharge their spores in succession and not *en masse*.

The upper surface of the pilei of the two species of Bolbitius which I have investigated is covered with a thin gelatinous cuticle which is formed from the swollen outermost walls of the exterior cells, and which can be torn away in strips from mature fruit-bodies (Fig. 37, D and E, p. 77). Such a gelatinous cuticle does not occur in connection with any of the thirty species of Coprinus which I have now examined. The upper surface of the pileus of a Coprinus is made up of (1) a continuous layer of thin-walled palisade cells some of which may be produced into unicellular hairs, or (2) loose spherical or elongated cells which constitute a mealy powder, or (3) masses of adherent cells which make up larger or smaller scales. It would therefore appear that the presence of a continuous gelatinous layer on the pileus of Bolbitius and its absence from Coprinus is a generic difference of some importance.

In their field characters the Bolbitii do bear a general resemblance to the Coprini. Among these characters may be mentioned :

(1) Thin flesh and relatively large gills.

(2) Relatively long, slender, hollow stipes.

(3) Delicacy and ephemeral nature of the fruit-bodies.

(4) Deliquescence of the gills and pileus-flesh.

(5) Fruit-bodies often coprophilous.

However, if instead of merely considering the more obvious characters we study the whole organisation of the fruit-bodies, taking into account both microscopic and macroscopic characters, then I think we must conclude that the two genera stand far apart. Their fruit-body mechanisms for the production and liberation of spores are essentially different, and one could not be converted into the other without important structural and physiological changes.

CHAPTER IV

THE ARMILLARIA SUB-TYPE ILLUSTRATED BY ARMILLARIA MELLEA, ETC.

Representative Species—Characters of the Armillaria Sub-type—*Armillaria mellea.* General Remarks—The Gills and the Hymenium—*Marasmius oreades*—*Russula ochroleuca*—*Amanita rubescens*—*Amanitopsis vaginata*—*Collybia radicata, C. velutipes, C. dryophila,* and *C. fusipes*—*Pluteus cervinus*—*Nolanea pascua*—Concluding Remarks

Representative Species.—The Armillaria Sub-type of fruit-body organisation is of very widespread occurrence among the Leucosporae and the Rhodosporae. To it belong such well-known fungi as the following :

Leucosporae		*Rhodosporae*
Armillaria mellea	Collybia dryophila	Pluteus cervinus
Amanita rubescens	Tricholoma nudum	Nolanea pascua
Amanitopsis vaginata	Mycena galericulata	
Marasmius oreades	Hygrophorus conicus	
Collybia velutipes	Russula cyanoxantha	
Collybia fusipes	Russula ochroleuca	
Collybia radicata	Lactarius quietus	

Whether or not any of the Ochrosporae belong to the Armillaria Sub-type remains to be decided by further investigation, but my observations point to the total exclusion of the genera Bolbitius, Galera, and Inocybe, and to the exclusion of at least a number of species of Cortinarius. Of the Porphyrosporae, the genera Stropharia, Psilocybe, and Psalliota are all included in the Panaeolus Sub-type ; and it is not unlikely that this will be the fate of all the species of this family. The Armillaria Sub-type, therefore, must

be considered as being confined, or almost confined, to the Leucosporae and the Rhodosporae. It must be noted, however, that not all the Leucosporae are included in this Sub-type ; for, as we have seen,[1] *Lepiota cepaestipes* belongs to the Psathyrella Sub-type and *Lepiota procera* has a hymenial organisation which, with the exception of the pigment in the spore-walls, is very similar to that of the Panaeolus Sub-type.

Characters of the Armillaria Sub-type. — The Armillaria Sub-type of fruit-body possesses all the general characters already described for the Aequi-hymeniiferous Type : the gills are wedge-shaped in cross-section and positively geotropic, the hymenium looks downwards to the earth, and every part of the hymenium produces and liberates spores during the whole period of spore-discharge.

The special characters of the Armillaria Sub-type are as follows :

(1) The whole structure of the fruit-body is relatively stout and suited to a persistence of the opened pileus for several days. In this respect, therefore, the Armillaria Sub-type resembles the Panaeolus Sub-type, but differs from the Psathyrella and the Bolbitius Sub-types. The pileus-flesh is often massive, the stipe mechanically resistant, and the gills firm.

(2) The spore-discharge period is several days in length and therefore relatively long. Here again, we have a contrast with the Psathyrella and Bolbitius Sub-types where, as we have seen, the spore-discharge period lasts for not more than about twenty-four hours. The actual number of days during which spores are discharged from the fruit-bodies of the characteristic species mentioned in the preceding Section has not been accurately measured. However, field mycologists well know that the fruit-bodies in question do not begin to decay until several days after they have opened. Now my own observations show that the spore-discharge period is continuous from its beginning, which is co-incident with the expansion of the pileus, to its end and that, as a rule, a fruit-body collapses a few hours after its last spores have been shed. The obvious inference is that persistent fruit-bodies have long spore-discharge periods. For *Armillaria mellea* I observed that spores were discharged from some fruit-bodies growing on a stump in the

[1] *Vide* Chapter I.

open for five successive days. At the end of this time, slugs entirely destroyed the fruit-bodies, and it was therefore impossible to make any further observations on the length of the spore-discharge period.

(3) The gills are not mottled in the Rhodosporae, and neither in this family nor in the Leucosporae are there irregular waves of development passing over the surface of the hymenium. In absence of mottling and wave development, the Armillaria Sub-type is markedly different from the Panaeolus Sub-type.

(4) On any small area of the hymenium the basidia are not assorted into definite groups of any kind. Basidia with ripe or nearly ripe spores are distantly separated from one another. In the middle of the areas between these most mature basidia are to be found younger basidia with less advanced spores, also distantly separated from one another. Still younger basidia with well-advanced sterigmata or very rudimentary spores may again occur in the middle of the spaces still left. These basidia, again, are distantly separated from one another. We thus find that, on any small area of the hymenium, there are spore-bearing basidia present in all stages of development, from those just discharging or about to discharge their spores to those with sterigmata just giving rise to spore-rudiments. The chief rule for the arrangement of all these basidia is that neighbouring basidia with spores of about the same age shall be distantly separated from one another. By this arrangement plenty of room is left for younger basidia to come up between older ones without any jostling.

(5) It is impossible to tell the number of generations of basidia coming up on any area of the hymenium, since the basidia at any one time are in all stages of development and not associated into age-groups. Here then we have a contrast with the other Sub-types which have been considered.

(6) The basidia are monomorphic, *i.e.* equally protuberant. In this respect our Sub-type agrees with the Panaeolus, the Bolbitius, and the Inocybe Sub-types, but differs from the Psathyrella Sub-type as well as from all the Coprinus Sub-types.

(7) There is no lateral crowding of the basidia with overlap of spores such as is found in the Psathyrella Sub-type and in the Coprinus Sub-types, but adjacent spore-bearing basidia are separated

by spaces greater than those in the Panaeolus Sub-type or in any other Sub-type whatsoever. The distant separation of basidia bearing spores of about equal age has already been insisted upon above.

(8) The paraphyses are relatively small and poorly developed. They are not joined laterally into a system of cells so as to form a pavement through which the basidia protrude ; but they are joined into strings of irregular character like those already described for the Panaeolus Sub-type. The paraphyses of the Armillaria Sub-type, therefore, contrast with those of the Psathyrella and the Bolbitius Sub-types as well as with those of all the Coprinus Sub-types.

From the above it will be noticed that the most striking feature of the organisation of the hymenium of the Armillaria Sub-type is the loose arrangement of the basidia which is of such a nature that *spore-bearing basidia of about the same age are distantly separated from one another*. It is important to grasp this essential character, for, when once grasped, if present in the hymenium of any fungus one is examining in surface view under the low power of the microscope, it can be detected at first glance.

Armillaria mellea. General Remarks.—*Armillaria mellea*, the Honey Fungus (Fig. 38, also Vol. II, Fig. 73, p. 203), is one of the best-known of the Agaricineae, for it is commonly found upon and about stumps in both Europe and North America as well as in many other parts of the world. It is characterised by its lignicolous habit, its yellowish-brown squamulose pileus, its somewhat decurrent white gills, and by having a stipe which is yellowish or honey-coloured at the base and floccose up to the spreading ring. The fungus is conspicuous in the woods in late summer and autumn, because it often occurs in dense tufts or clusters at the base of stumps or dead trees. To mycologists it is particularly interesting because, as Hartig [1] has shown, it is a facultative parasite and as such is sometimes responsible for the death of trees. Its mycelium has the remarkable property of exhibiting phosphorescence ; and, when woodmen fell infected trees in damp weather, the chips of wood and bark, as well as portions of the decorticated stumps and trunks, which are left upon the forest floor, not infrequently glow with such

[1] Robert Hartig, *Lehrbuch der Pflanzenkrankheiten*, Berlin, 1900, pp. 188–189.

intensity that the light from them can be seen at a distance of many yards. In the summer of 1917, I made a visit to Victoria Beach on the shore of Lake Winnipeg. On the day of my arrival, a dead tree was cut down in front of the bungalow where I was staying. The following night was a dark one and, on looking out from the verandah, I at once perceived a number of glowing chips of wood some ten yards distant. The light emitted was like that of feeble electric light. On picking up the chips, I found that the glow from them was just sufficient to enable me to read the time by my watch. The phosphorescence continued for three nights, after which it became too weak to be observed. The wood presented all the appearance of wood undergoing destruction by *Armillaria mellea*, and later in the season the fruit-bodies of the fungus were found in the neighbourhood, so that the identification of the mycelium was sufficiently established. The phosphorescence was so strong that it could be seen in the gloaming while a considerable amount of daylight still lingered from the afterglow of sunset. One of my friends collected the chips and laid them out on the ground so that the glow from them gave the impression of an immense, hollow-eyed, grinning skull which was intended to

FIG. 38.—*Armillaria mellea*, a cluster of fruit-bodies growing from the bark and wood of the trunk of *Pyrus Aucuparia*, in the autumn of 1902. The tree in 1921 was still flourishing. The parasite produced no more fruit-bodies after 1903. Photographed in Sutton Park, Warwickshire, by J. E. Titley. Much reduced.

affright belated wanderers returning home; but the besom of science has swept away so much superstition that the mycological ghost only afforded an occasion for merriment.

Armillaria mellea possesses a mycelium which is differentiated into three parts: (1) a loose branched part which penetrates through wood, etc., and brings about its destruction, (2) a *rhizomorpha subcorticalis* which occurs in the form of white sheets in or under the bark of a tree, and (3) a *rhizomorpha subterranea* which occurs as a series of branched and anastomosing, hollow, black, root-like strands, which are often to be found in spaces between loose bark and the wood or growing through the soil away from a tree-stump. Robert Hartig first showed the connection between the *rhizomorpha subterranea* and the fruit-bodies of *Armillaria mellea*.[1] Before his investigations the former was believed to be an independent fungus. Hartig discovered that strands of the *rhizomorpha subterranea* are able to grow through the soil from one root and enter another root by penetrating through the bark and cortex;[2] and he also found that they not infrequently give rise directly to the fruit-bodies.[3] We may therefore consider that the chief functions of the *rhizomorpha subterranea* are two: (1) to spread the fungus locally by growing through the soil from one root to another, and (2) to produce fruit-bodies in favourable positions at the surface of the soil.

According to Robert Hartig, *Armillaria mellea* is able to kill certain coniferous trees, such as *Picea excelsa*, and under certain conditions can live as a parasite on woody Dicotyledons such as species of Prunus, Amygdalus, and Acer.[4] I myself have made an observation in this connection which seems worth recording. In 1901, at Sutton Park, near Birmingham, England, there was a large

[1] Robert Hartig, *Wichtige Krankheiten der Waldbäume*, Berlin, 1874, pp. 22–27.

[2] O. Brefeld (*Untersuchungen*, Heft III, 1877, pp. 150–151) brought the tips of rhizomorphs grown in pure culture into contact with freshly-dug sound roots of *Pinus sylvestris* and observed that penetration through the bark took 1–2 days. After penetration, the mycelium grew rapidly under the bark, making its way through the cambial region.

[3] Robert Hartig, *loc. cit.*

[4] *Ibid.*; also Robert Hartig, *Lehrbuch der Pflanzenkrankheiten*, Berlin, 1900, pp. 188–189. W. E. Hiley (*The Fungal Diseases of the Common Larch*, Oxford, 1919, pp. 144–145) asserts that, in Europe, more trees die from attack by *Armillaria mellea* than through any other parasitic agent.

wild Mountain Ash (*Pyrus Aucuparia*) upon the base of the trunk of which a number of fruit-bodies of the Honey Fungus appeared. In 1902 a still larger number of fruit-bodies came to maturity upon the tree, and the photograph which is here reproduced was then taken (Fig. 38 ; *cf.* also Fig. 30, Vol. I, p. 78). A small piece of the bark was removed and was found to be phosphorescent in the dark. The next year only a few fruit-bodies developed, and thereafter no more at all. The tree in 1921, *i.e.* after a lapse of twenty years, was still flourishing. It thus appears that the mycelium of the fungus, after succeeding in entering the tree, spreading beneath the bark, and maintaining its vitality for at least three years, died and left its host-plant victorious in the struggle. One might have expected that the parasite would complete the destruction of its victim. The reason for this failure I am not able to suggest.

Armillaria mellea can be grown in pure cultures without difficulty. Brefeld [1] (1877) germinated spores in a drop of plum decoction on a glass slide and then transferred the resulting mycelium to a large mass of plum decoction contained in a crystallising dish. He soon obtained numerous rhizomorphs but did not succeed in raising any fruit-bodies. Molisch [2] (1904), using a Petri dish, sowed spores in a plum decoction solidified with agar and then transferred the mycelium to plum decoction or wet bread-mash contained in an Erlenmeyer flask. Like Brefeld, he soon obtained rhizomorphs, but, on the bread-mash, at the end of two months from sowing the spores, he also obtained several fruit-bodies. Both Brefeld and Molisch observed that the mycelium in their cultures was splendidly luminescent.

Hans Kniep [3] obtained separate mycelia each of which originated from a single spore ; and, after 2–4 weeks, he found that, although the cultures exhibited no trace of fruit-bodies, yet the aerial hyphae had given rise directly to basidia bearing basidiospores. This production of scattered basidia on a mycelium of monosporous origin

[1] O. Brefeld, *Untersuchungen*, Heft III, Leipzig, 1877, pp. 141–146.

[2] H. Molisch, *Leuchtende Pflanzen*, Jena, 1904, pp. 36–38.

[3] H. Kniep, "Über das Auftreten von Basidien im einkernigen Mycel von *Armillaria mellea*," *Zeitschrift für Botanik*, Jahrg. III, 1911, pp. 529–553.

is a curious phenomenon which has also been observed by Holtermann [1] in cultures of *Polyporus bogoriensis*.

Kniep [2] observed that while a young basidium on the gills of a normal fruit-body of *Armillaria mellea* contains two nuclei, a young basidium arising directly on a mycelium of monosporous origin contains only one nucleus. This difference may be readily explained if we assume that *A. mellea*, like *A. mucida*,[3] is heterothallic. In all probability, in nature, each mycelium which gives rise to a fruit-body arises from the union of two monosporous mycelia of opposite sex. Hence the mycelium comes to have two nuclei in each cell and, ultimately, the fruit-body two nuclei in each young basidium. On the other hand, a mycelium grown from a single spore is necessarily unisexual (haploid) and therefore has but one nucleus in each young basidium produced directly upon the aerial hyphae. Experiment with other heterothallic Hymenomycetes, *e.g. Coprinus fimetarius*, *C. lagopus*, *Collybia velutipes*, and *Schizophyllum commune*, has shown that, while a secondary (diploid) mycelium produced by the fusion of two primary (haploid) mycelia of opposite sex produces fruit-bodies of normal appearance and vigour, single primary mycelia remain quite sterile, or produce imperfect fruit-bodies, or produce fruit-bodies of normal form only occasionally and usually after a much longer time than secondary mycelia.[4] In *Armillaria mellea* the secondary mycelia produce large fruit-bodies with stipes,

[1] C. Holtermann, *Mycologische Untersuchungen aus den Tropen*, Berlin, 1908, pp. 94–95, Taf. IX, Fig. 8.

[2] H. Kniep, *loc. cit.*, Taf. III.

[3] H. Kniep, "Über morphologische und physiologische Geschlechtsdifferenzierung," *Verhandlung der Physikal.-med. Gesellschaft zu Würzburg*, 1919, pp. 12–13.

[4] Mlle Bensaude asserts that monosporous mycelia of *Coprinus fimetarius* are completely sterile. Miss Mounce and W. F. Hanna, in my laboratory, have observed that, while monosporous mycelia of *C. lagopus* always produce fruit-bodies, these fruit-bodies have white or whitish gills and produce relatively few spores or no spores at all. When spores are produced they are not shot from their sterigmata. Miss Dorothy Newton, another worker in my laboratory, observed that of several monosporous mycelia of *Collybia velutipes* only one produced a fruit-body. The fruit-body had a normal appearance but its spores would not germinate. Miss E. M. Wakefield and Hans Kniep have observed that monosporous mycelia of *Schizophyllum commune* are usually, although not always, quite sterile. For references to the papers of Bensaude, Kniep, and Mounce, *vide* these *Researches*, vol. ii, 1922, p. 395.

pilei, and gills, whilst a primary mycelium grown by itself appears to have such weak fruiting powers that it gives rise not to normal massive fruit-bodies but simply to isolated scattered basidia.

When fruit-bodies of *Armillaria mellea* occur in large clusters, collectively they may give off such vast numbers of basidiospores that the escaping spore-clouds, under favourable conditions of diffuse daylight, may be perceived even with the naked eye.[1]

The fruit-bodies of *Armillaria mellea* are edible, but mycophagists dislike their flavour and do not highly recommend them. Squirrels[2] and certain slugs[3] eat them with avidity.

In the United States of America there is an agaric known as *Clitocybe monadelpha* Morg. which resembles *Armillaria mellea* in its honey colour and scaliness, etc., but is distinguished from it by the absence of the annulus, by the more decurrent gills, and by the solidity of its stipe.[4] Miss C. A. Richards[5] has recently discovered that *Clitocybe monadelpha*, in its rhizomorphs, in its mycelial sheets, in its mode of rotting wood, and in cultures obtained from its rhizomorphs, very closely resembles *Armillaria mellea*. Particularly on account of the striking similarity of the rhizomorphs I am inclined to regard *Clitocybe monadelpha* as merely a ringless form of *Armillaria mellea*. In Europe *Clitocybe tabescens*, beautifully illustrated in colours by Boudier,[6] appears to be merely another ringless form of *Armillaria mellea* and it is so regarded by Rea.[7] Kauffman states that *Clitocybe monadelpha* "is apparently the American form of *C. tabescens*."[8]

The Gills and the Hymenium.—The gills of *Armillaria mellea* in cross-section have the usual wedge shape so characteristic of the Aequi-hymeniiferae and are positively geotropic, so that their

[1] *Vide* these *Researches*, vol. ii, 1922, pp. 100–103.

[2] *Ibid.*, pp. 197–199.

[3] Slugs, probably *Limax maximus*, so voraciously attacked the *A. mellea* fruit-bodies used for determining the length of the spore-fall period that my observations were brought to a premature end.

[4] C. McIlvaine and R. K. Macadam, *One Thousand American Fungi*, Indianapolis, 1902, p. 89, Plate XXVII.

[5] C. Audrey Richards, *in litt.*

[6] E. Boudier, *Icones Mycologicae*, Paris, 1905–1910, T. I, Plate LXI.

[7] C. Rea, *British Basidiomycetae*, Cambridge, 1922, p. 110.

[8] C. H. Kauffman, *The Agaricaceae of Michigan*, Lansing, vol. i, 1918, p. 723.

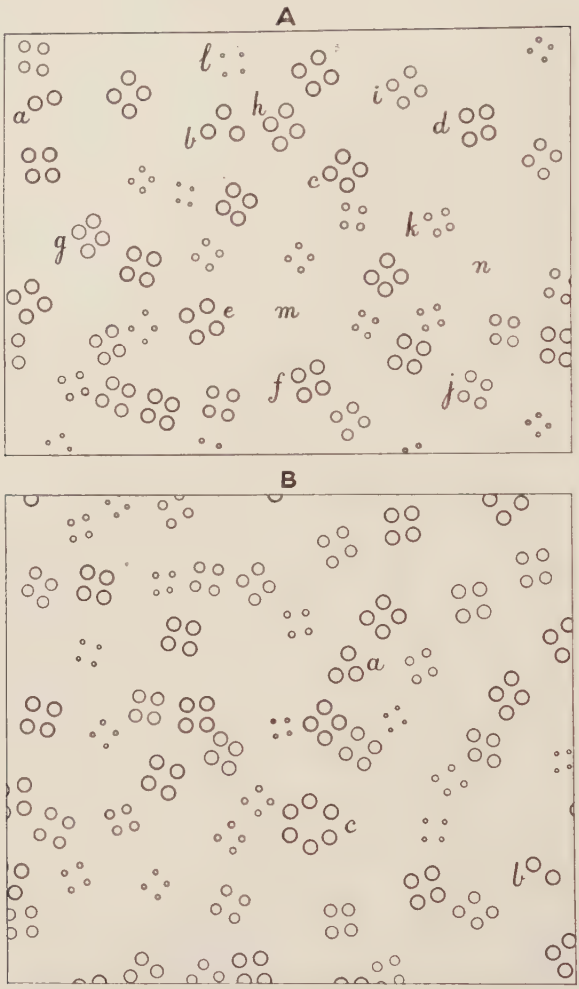


Fig. 39. —*Armillaria mellea.* Surface views of the hymenium showing the positions of the spores ; A, drawn during the first day of the spore-discharge period ; B, drawn during the second day. In A : at *a* two spores, and at *b* one spore, have just been shot away ; at *c*, *d*, *e*, and *f*, etc., the spores are of full size and will be discharged within a few minutes ; at *g* and *h*, the spores have just attained full size ; at *i* and *j*, the spores are about half-grown ; at *k*, the spores are very young ; and at *l*, the spores are only just beginning to show on the ends of their sterigmata ; in each of the spaces *m* and *n* a basidium has just shot away its four spores, and here other basidia will shortly begin to develop spores. The wide separation of basidia bearing spores of about the same age—a chief character of the Armillaria Sub-type—is to be noticed. In B, which is similar to A : at *a* is a basidium which has just shot away a single spore and at *b* a similar basidium which has just shot away two spores ; at *c* is an abnormal basidium of twice the usual size which has six spores instead of four. Magnification, 330.

hymenial surfaces come to look downwards to the earth under normal conditions. The period of spore-discharge continues for at least five days and possibly for a longer time. As already recorded, my observations in this connection were cut short by slugs (p. 85). The pileus-flesh is thick and firm, and the stipe stout. The fruit-body as a whole is both structurally and functionally a relatively persistent one.

The hymenium is composed of basidia and paraphyses only. Cystidia are lacking on the sides of the gills. The basidia are relatively long, thin, and closely packed. The paraphyses are numerous and, in general, have the same distribution and importance as in *Panaeolus campanulatus*. The hymenial elements arise on branching cells which spring from the trama and constitute the subhymenium.

The chief point of interest in the hymenium is the distribution of the spore-bearing basidia, for upon this depends, as we have seen, the differentiation of the Armillaria Sub-type from the other Sub-types of the Aequi-hymeniiferae. This distribution on the first day of spore-discharge is shown in Fig. 39 at A, and for the second day in the same figure at B. The general appearance of the spore-bearing basidia remained about the same for the first five days of the spore-discharge period. An examination of the two drawings, A and B, permits one to perceive the loose arrangement of the hymenium : basidia of about the same age, as regards the development of their spores, are not crowded together in groups as in Panaeolus, but are scattered so that, as a rule, they are isolated from one another or, occasionally, two are near one another. Basidia with spores in their earliest state of development are isolated from one another in a similar manner, and so on for any series of spore-bearing basidia of about the same age. The reader should compare Fig. 39 with the corresponding figures for the Panaeolus (Vol. II, pp. 257 and 258), the Psathyrella (pp. 14 and 50), and the Bolbitius Sub-types (pp. 69 and 73). The figures for the first two of these Sub-types afford striking contrasts with Fig. 39 ; but that for the Bolbitius Sub-type is somewhat similar. In a Bolbitius, however, we have very large paraphyses united into a definite system instead of thin inconspicuous cells not so united. Moreover,

in a Bolbitius, the basidia are not nearly so numerous and shed spores for only about twenty-four hours.

FIG. 40.—*Marasmius oreades*, the Fairy-Ring Fungus. A characteristic group of fruit-bodies coming up in short grass. Photographed in Yorkshire by A. E. Peck. Natural size.

The rate of development of the spores on individual basidia was observed by means of the compressor-cell method described in

Volume II.[1] In six basidia it was found that the period of time elapsing from the first formation of the spores as tiny rudiments to the discharge of the first spore was on the average about one hour and thirty minutes. The observations were made in the middle of the second day of the spore-discharge period. Let us consider the appearance of the hymenium as it is at any particular moment, such as is represented by the *camera-lucida* drawing given in Fig. 39, A. After about one hour and a half this appearance will have completely changed, for all the spores, even those which were at first nothing but tiny rudiments, will have been discharged. If we assume, as I think we are justified in doing, that the rate of development of the spores on individual basidia remains fairly constant for a period of twenty-four hours, then in this time there would be sixteen complete changes in the appearance of the hymenium so far as the spore-picture is concerned. With the lengthening of the period of spore-discharge to five days the number of changes in the spore-picture would be proportionately increased. Even should the rate of development of the spores of individual basidia slow down somewhat toward the latter part of the spore-discharge period, as I suspect it does, we can yet be safe in believing that the number of complete changes in the spore-picture of the hymenium on any one particular area is very large, in round figures probably exceeding fifty. It may be added that the number of basidia developing spores at one and

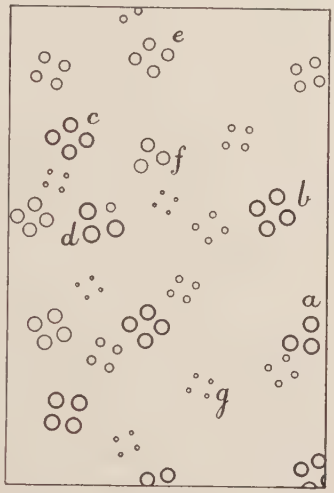


FIG. 41.—*Marasmius oreades.* The hymenium, about the middle of the spore-discharge period, seen in surface view, showing the spores only. The basidia which, as regards their spores, are in about the same stage of development are well separated from one another. At *a* is a basidium which has just shot away its first spore; *b*, *c*, two basidia with nearly ripe spores; *d*, an abnormal basidium with three nearly ripe spores and one aborted spore; *e*, four partly grown spores; *f*, a basidium having three spores only; *g*, four spores just coming into existence on the ends of their sterigmata. Magnification, 330.

[1] *Researches on Fungi*, vol. ii, 1922, pp. 43–46.

the same time on any unit area of the hymenium probably decreases with the gradual exhaustion of the hymenium, as was observed in *Panaeolus campanulatus* and certain other Hymenomycetes.

Marasmius oreades.—This fungus (Fig. 40), often called the Fairy-Ring Fungus, is very common in Europe on grassy meadows and lawns where it forms the well-known "fairy rings." Like other Marasmii, it is able to withstand desiccation for some days or weeks without loss of vitality. After a drought, when rain has come, it quickly absorbs water through the surface of its pileus and within a few hours begins once more to shed a cloud of spores. In the general structure of the fruit-body as a whole, in its persistence, and in the arrangement of the basidia in the hymenium *Marasmius oreades* is organised in perfect accordance with the Armillaria Sub-type.

For our present purpose it is only necessary to describe the appearance of the spores of the spore-bearing basidia of the hymenium when this is seen in face view, for this gives the clue to the arrangement of the basidia as a whole in respect to one another both in space and time. A surface view of a piece of living hymenium actively producing and discharging spores is shown in Fig. 41. It will be seen at a glance that the arrangement of the spore-bearing basidia is like that described for *Armillaria mellea*. The basidia with the most rudimentary spores are well separated from one another and not compactly arranged in groups. The same is true of the basidia which bear full-sized spores, and of those which bear spores of an intermediate size. The rule of wide separation of basidia bearing spores of about equal age is well kept from day to day throughout the whole period of spore-discharge.

Russula ochroleuca.—Another excellent example of an Agaric exhibiting the loose type of hymenium characteristic of the Armillaria Sub-type is provided by *Russula ochroleuca*, a species of which specimens were collected in the Midlands of England. In order to bring out the rule, here again exhibited, that basidia bearing spores about equally advanced in development are distantly separated from one another and not packed together in little groups, let us regard the spores in the accompanying illustration of a surface view of the hymenium (Fig. 42) as roughly of four different ages :

(1) ripe or almost ripe spores, like those at *a* and *b* ; (2) spores which have just attained full size or are almost of full size, like those at *c*, *d*, *g*, and *h* ; (3) very young spores of the size of those at *f*, or somewhat larger ; and (4) spores just beginning to form on the ends of

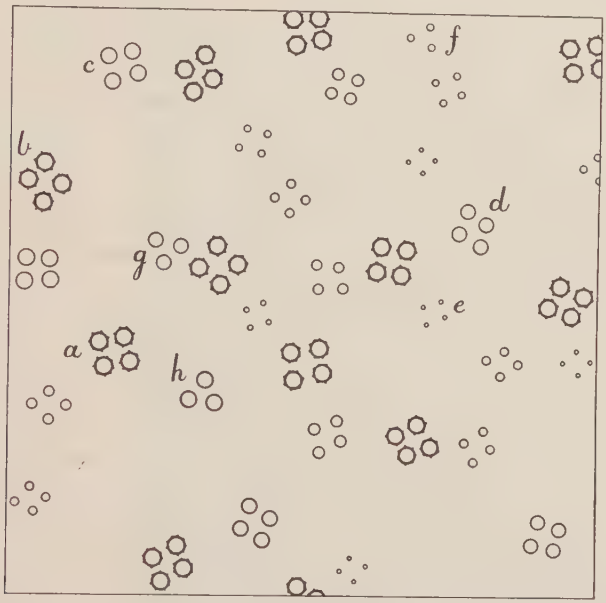


FIG. 42.—*Russula ochroleuca.* Surface view of the hymenium showing the spores, on the first day of the spore-discharge period. The drawing was made with the *camera lucida*, but the roughnesses on the walls of the almost ripe spores were added diagrammatically. *a* and *b*, two of the oldest basidia with ripe or almost ripe spores ; *c*, four spores which have almost attained full size but which still have smooth walls ; *d*, half-grown spores ; *e*, spores which are just beginning to form on the ends of their sterigmata ; *f*, *g*, and *h*, abnormal trisporous basidia. Magnification, 330.

the sterigmata, like those at *e*. If one considers the position of the basidia bearing spores of any one age, one at once perceives that the individual basidia are well isolated from one another.

Amanita rubescens.—This well-known Amanita (Vol. II, Figs. 135, 136, pp. 378, 379) clearly belongs to the Armillaria Sub-type of fruit-body. A glance at the surface view of the hymenium, illustrated in Fig. 43, A and B, shows that the basidia which bear spores of about the same age are widely separated from one another.

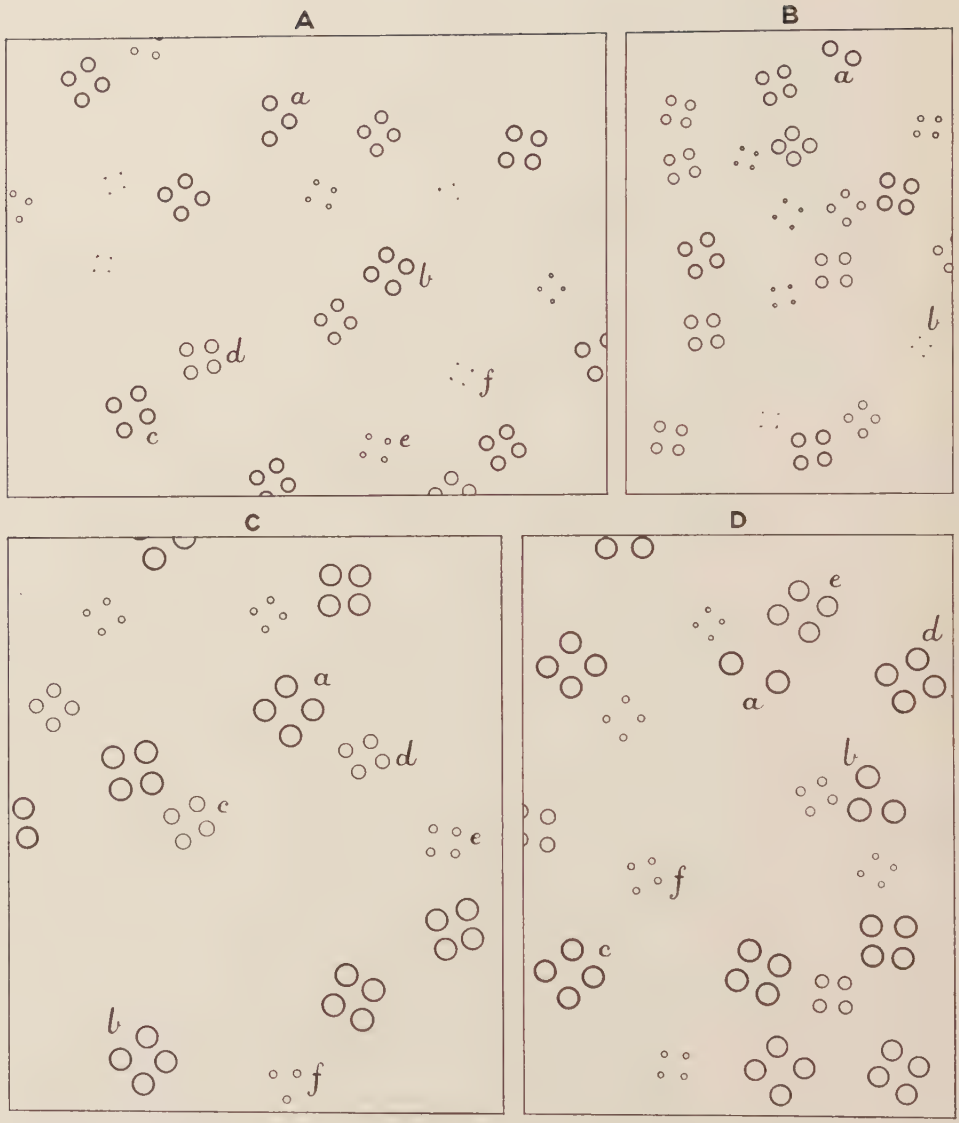


FIG. 43.—The loose hymenium of the Armillaria Sub-type. A and B, surface views of two areas of the hymenium of *Amanita rubescens* in the middle of the spore-discharge period, showing spores. C and D, two similar areas for *Amanitopsis vaginata*. In A: at *a*, a basidium which has discharged its first spore; at *b* and *c*, two basidia with nearly ripe spores; at *d*, half-grown spores; at *e*, very rudimentary spores; at *f*, four sterigmata about to develop spores. In B: at *a*, a basidium which has just discharged two of its spores; at *b*, four sterigmata about to develop spores. In C: at *a* and *b*, nearly ripe spores; at *c*, *d*, *e*, successively younger spores; at *f*, a basidium with three spores only. In D: at *a*, a basidium which has just shot away two of its spores; at *b*, a basidium which has just shot away its first spore; at *c* and *d*, basidia with nearly ripe spores; at *e*, a basidium with spores almost of full size; at *f*, a basidium just beginning to develop spores. Magnification, 330.

The new spores which arise as tiny rudiments are evidently being produced here and there in the middle of spaces not occupied by older spore-bearing basidia. Every series of basidia which at any particular time are giving rise to rudiments of spores has the same scattered arrangement. Hence, of course, it follows that, in general, basidia bearing spores of about the same age are scattered over the hymenium and not gathered into compact groups. This loose arrangement of the basidia was found to be characteristic of pilei which were just expanding, pilei which were well expanded, and pilei which were older and expanded to the full limit.

FIG. 44.—A fruit-body of *Amanitopsis vaginata*. The pileus is fully expanded and shedding spores. The stipe is surrounded at its base by the volva and bears a few excreted water-drops. Photographed at Sutton Park, Warwickshire, by J. E. Titley. About ½ natural size. The height was 4 inches.

Amanitopsis vaginata.—The fruit-body of this common and beautiful species differs from that of *Amanita rubescens* and the Amanitae generally in that, although possessing a volva at the base of the stipe, it lacks an annulus (Fig. 44). The ensheathing volva is usually half-hidden in the ground, but in a fruit-body which has been carefully gathered it forms a conspicuous structure that is large, upright, free, lax, and membranous (Fig. 45). The wedge shape of the gills in transverse section, the slightly downward-

looking position of the hymenium, the form of the basidium, the mode of attachment of a spore to its sterigma, and some sporabolic trajectories are shown in Volume I, Fig. 56 (p. 165). The spores are large and globose and each one comes to contain one or more bulky oil-drops or *guttae*. The presence of this oil helps to account for the fact that the specific gravity of the spores only just exceeds that of water.[1] It was the determination of the radius, the density, and the terminal rate of fall in still air of the spores of *Amanitopsis vaginata* which enabled me to make the first rough test of the accuracy of Stokes' Law for the fall of minute spheres in a gaseous medium.[2]

FIG. 45.—*Amanitopsis vaginata*. The fruit-body shown in Fig. 44 removed from the ground and standing upon its pileus. The volva surrounding the base of the stipe is now well displayed. Photographed by J. E. Titley. About ½ natural size.

There is no essential difference between the arrangement of the spores in *Amanitopsis vaginata* (Fig. 43, C and D) and in *Amanita rubescens*. It is interesting to note the resemblance of the structure of the hymenium in species of two genera which are generally regarded as being closely related to one another.

Collybia radicata, C. velutipes, C. dryophila, and C. fusipes.—All these four fungi have the loose type of hymenium characteristic of the Armillaria Sub-type. The accompanying illustration (Fig. 46) shows the spore-picture for a piece of the hymenium of *Collybia radicata* when seen in surface view. The scattered arrangement of the basidia is very marked. There is evidently no massing of basidia of like ages into compact groups, so that once more we have a striking contrast with the appearance of the hymenium of *Panaeolus campanulatus*. The fact that four species of Collybia all possess the

[1] *Researches on Fungi*, vol. i, 1909, pp. 154–156. [2] *Ibid.*, pp. 164–173.

loose type of hymenium suggests that this kind of organisation is characteristic for the whole genus. The rate of development of the spores on individual basidia is a rapid one in Collybia, for the time from the first appearance of the rudiments of the four spores up to the moment of discharge of the first was observed to be as follows : for *Collybia velutipes* 47 minutes, for *C. radicata* 1 hour and 30 minutes, and for *C. dryophila* (incomplete observations) between 1 hour and 1 hour 30 minutes.[1]

Some other details of the structure of the hymenium of *Collybia radicata* are indicated in Fig. 46, B, C, and D. B is a surface view of the hymenium showing (1) basidium-bodies, *b*, containing abundant granular protoplasm, and (2) somewhat smaller paraphyses, *p*, with relatively scanty protoplasm appressed to the cell-walls and large central vacuoles. C represents a cross-section through the upper part of the hymenium showing the external appearance of the various elements : the least prominent cells, *d*, consist of very young basidia and of paraphyses ; *c* is an older basidium which has become protuberant ; *b* is a basidium which has developed its sterigmata and has just begun to develop spores, the spore-rudiments being less than 10 minutes old ; *a* is a basidium with full-grown spores, the spores being between 35 minutes and 90 minutes old ;[2] *e* is a cystidium with a rounded head. D represents a cross-section through the hymenium showing a few elements with their contents : *a* and *b* are young basidia filled with granular protoplasm ; *c* is an older basidium, elongating and about to form sterigmata, also filled with granular protoplasm ; *d* is a fully protuberant basidium which bears four full-size spores into which the protoplasm of the basidium-body is still passing through the sterigmata, possibly owing to the growth of the vacuole *v* and the pressure of the cell-sap ; *e*, *f*, and *g* are paraphyses, somewhat smaller than the young basidia and distinguished by their large vacuoles and diminishing protoplasmic contents ; *h* is a cystidium with a bluntly rounded end and vacuolated protoplasm.

The drawings B, C, and D in Fig. 46 clearly indicate the two

[1] *Researches on Fungi*, vol. ii, 1922, p. 44.

[2] The spores of *Collybia radicata* require about 35 minutes to grow to full size and are shot away when they are about 1 hour and 30 minutes old. *Vide* these *Researches*, vol. ii, 1922, p. 54.

following characteristics of the hymenium of the Armillaria Sub-type: (1) the monomorphism of the basidia which contrasts with

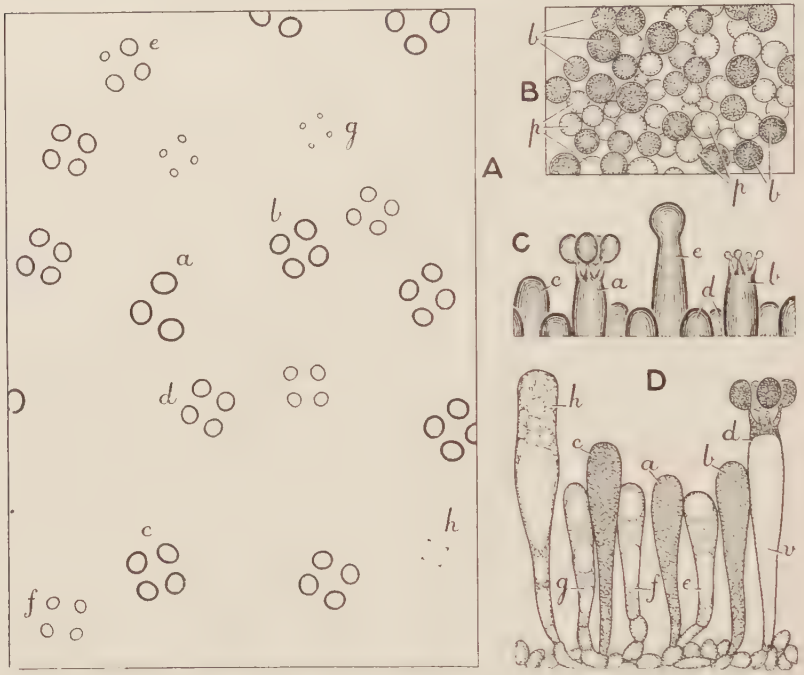


FIG. 46.—*Collybia radicata*. The loose hymenium of the Armillaria Sub-type. A, surface view of the hymenium in the middle of the spore-discharge period, showing the spores; at *a*, one of the four spores has just been discharged; at *b* and *c*, almost ripe spores; at *d*, a basidium with spores not yet of full size; at *e*, a similar basidium, but one spore is aborted; at *f* and *g*, very young spores; at *h*, four sterigmata about to develop spores. B, a surface view of the hymenium showing: basidium-bodies, *b*, containing abundant granular protoplasm, and paraphyses, *p*, containing large vacuoles. C, a cross-section through the upper part of the hymenium: *a*, a basidium bearing spores; *b*, a basidium just beginning to develop spores; *c*, a protuberant basidium which will soon develop sterigmata and spores; *d*, younger basidia and paraphyses; *e*, a cystidium with a rounded head. D, a cross-section through a hymenium showing a few elements with their contents: *a* and *b*, young basidia filled with granular protoplasm; *c*, an older basidium, elongating and about to form sterigmata, now filled with granular protoplasm; *d*, a basidium with four full-size spores, the protoplasm is still passing into the spores through the open sterigmata and, in consequence, the vacuole, *v*, is still growing in size; *e*, *f* and *g*, paraphyses containing large vacuoles and diminishing protoplasmic contents; *h*, a cystidium, only slightly swollen at its free end, containing vacuolated protoplasm. Magnification, 293.

the polymorphism of the basidia in the Psathyrella Sub-type and in the Coprinus Sub-types, and (2) the fact that the paraphyses, unlike those of the Psathyrella, Bolbitius, and Coprinus Sub-types,

are not welded together into a firm and continuous membrane but are somewhat loosely associated with one another.

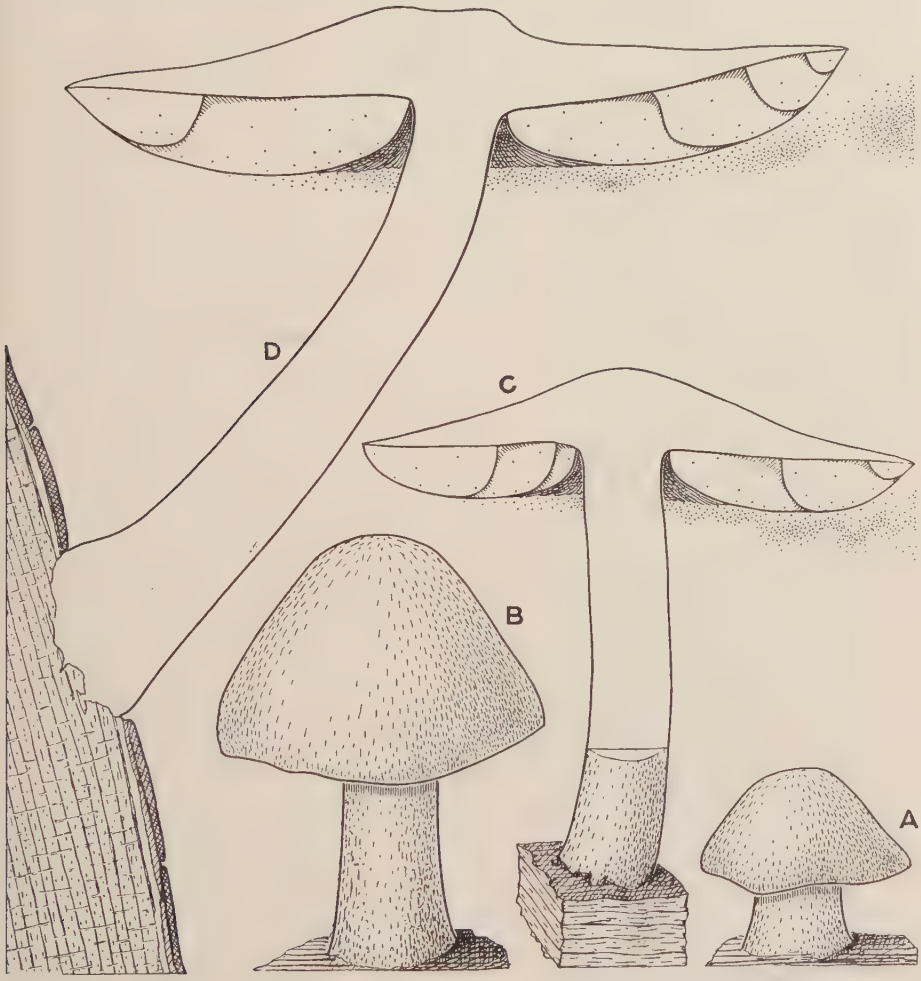
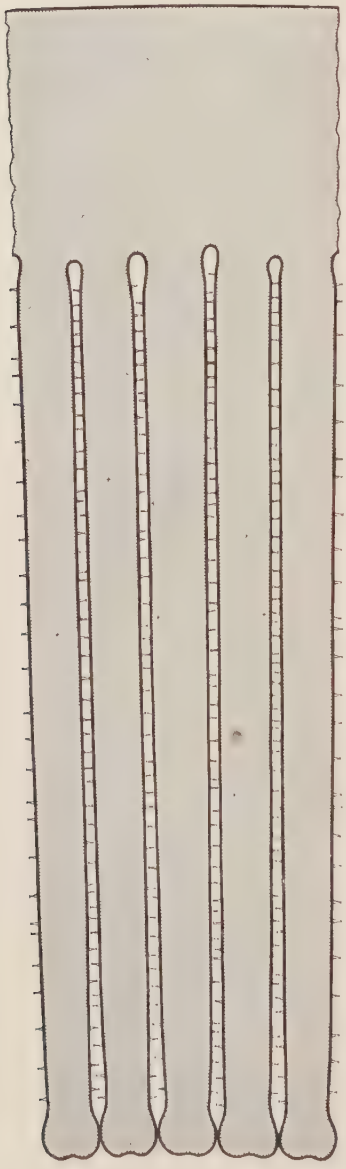


FIG. 47.—*Pluteus cervinus.* Fruit-bodies attached to the wood of a rotten stump of *Acer pseudoplatanus.* A, a young fruit-body in which the cystidia are developing but not sterigmata. B, an older fruit-body in which the cystidia are fully developed and cross the interlamellar spaces, but in which as yet there are neither spores nor even sterigmata. C, a fully expanded fruit-body, seated on the top of the stump, shedding spores which are represented as being carried off by a slight breeze. D, a similar fruit-body growing from the side of the stump. Natural size.

Pluteus cervinus.—*Pluteus cervinus* is a pink-spored fungus of considerable size, often found coming up upon old stumps and rotting

logs in both Europe and America (Fig. 47). It frequently appears in the woods in Manitoba. Its hymenium attracted attention one hundred years ago owing to the fact that it is beset with barbed cystidia. These curious structures were first described and illustrated by Ditmar in 1817 in Sturm's *Deutschlands Flora*.



I have investigated the structure of the fruit-body of *Pluteus cervinus* in some detail, and I now propose to give an account of the chief facts observed.

If one examines the gills of a very young fruit-body (A in Fig. 47) in face view and transverse section (Fig. 49, A and B), one finds that the cystidia (*c*) can already be distinguished from the basidia and paraphyses by their much larger size and greater protuberance; and, at this stage of development, it is evident that the cystidia are growing rapidly at their apices in such a way as to extend into, and more or less cross, the very narrow interlamellar spaces (*i* in B).

In an older fruit-body, just before the expansion of the pileus (B in Fig. 47), the gills are still packed closely together. In a cross-section (Fig. 48) they are seen to be slightly

FIG. 48.—*Pluteus cervinus*. Transverse section through the pileus and gills of the unexpanded fruit-body, B, shown in Fig. 47. The gills have slight flanges at their free ends. As yet all the basidia are immature, for none of them have developed any spores or sterigmata; but the cystidia are full-grown. The cystidia project across the interlamellar spaces and so prevent opposing hymenial surfaces from being pressed together. Magnification, 23.

wedge-shaped and to have a small flange at their free edge directed toward the stipe. The interlamellar spaces have become slightly widened and are now crossed by numerous fully-developed barbed cystidia which everywhere beset the hymenium (Fig. 48). The microscopic appearance of a gill at this stage of development is shown in face view and in cross-section in Fig. 49 at C and D respectively. A study of these two drawings shows that of the three classes of elements which make up the hymenium, namely, cystidia, basidia, and paraphyses, the first-named are the first to attain maturity: the cystidia have already grown to their full length and have developed their hooks before any rudiments of spores or even of sterigmata have begun to appear on the basidia.

As a pileus expands, the gills are raised into more or less horizontal positions (C and D in Fig. 47) and, at the same time, they separate from one another, so that the interlamellar spaces increase greatly in width. A vertical section through parts of two gills and an intervening interlamellar space is shown in Fig. 49 at E. At this stage of development the cystidia no longer cross the interlamellar spaces but merely project from the hymenium like so many pegs, and the basidia are freely discharging their spores.

The spore-picture presented by a surface view of the hymenium when shedding spores (Fig. 50, A and B) is similar to that already several times described for the Armillaria Sub-type. The only novel point is the presence of the barbed cystidia. As in *Armillaria mellea*, *Marasmius oreades*, etc., on any very small area of the hymenium one sees spores in all stages of development, and one can observe that basidia bearing spores of about equal age in development are usually well separated from one another.

A transverse section through a gill (Fig. 51) shows the hymenium, subhymenium, and trama. The hymenium is made up of cystidia, basidia, and paraphyses. The cystidia (*a* and *b*) often have a deep-seated origin, for they can be observed arising from the outer cells of the trama just below the subhymenium. Each cystidium has a fusiform shaft and a free end which expands into a series of hooks. The hooks on a single cystidium are usually three or four in number, but may be two only or as many as five (Figs. 49, C, and 50, A). Their shape is also variable. It often happens that a hook is bifid,

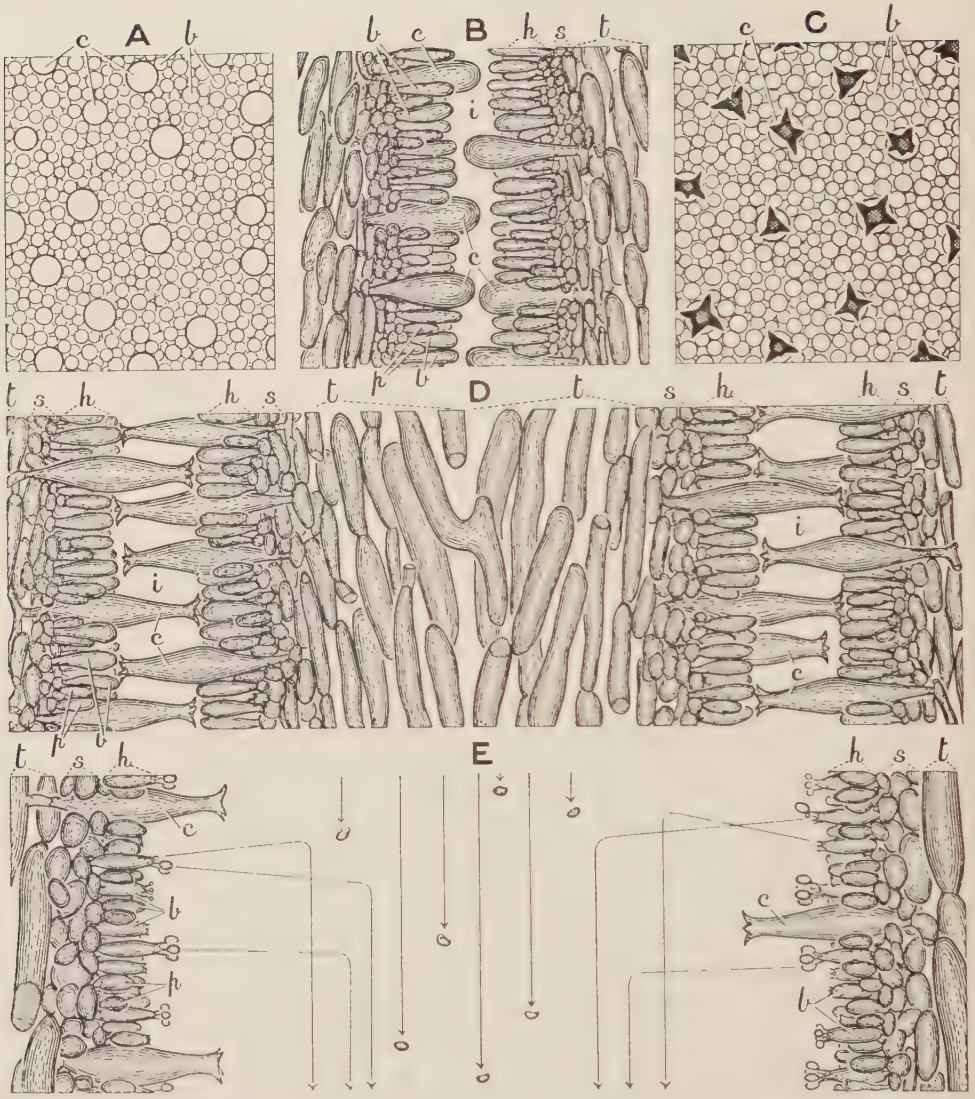


FIG. 49.—*Pluteus cervinus.* A, surface view of a gill taken from a very young fruit-body (A in Fig. 47) : *c*, cystidia still with rounded apices ; *b*, basidia protruding beyond, and thereby largely covering, the paraphyses. B, a vertical transverse section through parts of two gills of the same fruit-body (A in Fig. 47) : *i*, the interlamellar space between the gills ; *h*, the hymenium ; *s*, the subhymenium ; *t*, the trama ; *c*, young cystidia ; *b*, basidia ; *p*, paraphyses. C, surface view of a gill of a fruit-body shortly before the opening of the pileus (B in Fig. 47) : *c*, cystidia, already with apical prongs and fully developed ; *b*, basidia. D, a vertical transverse section through three gills of the same fruit-body (B in Fig. 47) : *h*, the hymenium ; *s*, the sub-hymenium ; *t*, the trama ; *i i*, interlamellar spaces into which the cystidia, *c*, project (*cf.* Fig. 48) ; *b*, basidia ; *p*, paraphyses. E, a vertical transverse section through two gills of a fully expanded fruit-body (C or D in Fig. 47) during the spore-discharge period ; in the centre a wide interlamellar space into which spores are being discharged ; *h*, the hymenium ; *s*, the subhymenium ; *t*, the trama ; *c*, cystidia now projecting freely into the widened interlamellar space ; *b*, basidia ; *p*, paraphyses ; a basidium near the top of the right hymenium is about to discharge its last spore, as is indicated by the size of the drop of fluid already excreted at the spore-hilum. The arrows directed away from the hymenium indicate the trajectories described by some of the spores when shot violently from their sterigmata. The spores falling in the middle of the interlamellar space have been shot away from the hymenium on parts of the gills which are higher than those here shown : two of them (arrows Nos. 2 and 6 from the left) still retain the drop of fluid which was discharged with them ; from other spores the drop has already evaporated ; other spores (arrows 7 and 9 from the left) are already drying up and becoming boat-shaped. Magnification, 293.

in which case the two minor hooks thus produced may be equal in size or one may be larger than the other, the inequality exhibiting all gradations. The wall of a cystidium is thin below but thickens where the shaft protrudes into an interlamellar space, and it is thickest at the hooks (Fig. 51, *a* and *b*). Treated with chlor-zinc iodine, the thicker parts of the wall swell up and become violet-red. At the same time strings or discs, which cross the free end of the

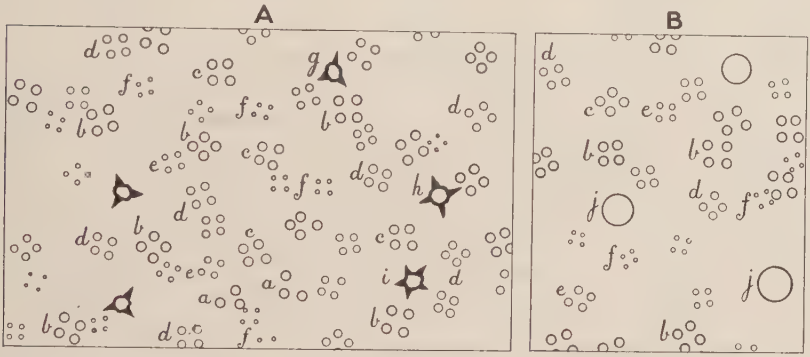


FIG. 50.—*Pluteus cervinus.* A, surface view of a gill during the spore-discharge period, showing the distribution of the spore-bearing basidia, as indicated by their spores, and of the cystidia, as indicated by their branched apices: *a a*, basidia from which a spore has just been discharged; *b b*, basidia about to discharge their spores; *c c*, basidia with spores that have just attained full size; *d d*, basidia with spores about two-thirds the full size; *e*, basidia with very rudimentary spores; *f f*, basidia with spores just coming into existence on the ends of the sterigmata; *g*, *h*, and *i*, cystidia with 3, 4, and 5 prongs respectively. B, another similar drawing but with the cystidia, *j*, represented by median sections instead of by their apices; letters *b–f*, as in A. Drawn with the *camera lucida*. Magnification, 293.

cystidium and at first resemble cell-walls, stain yellow and thus indicate that they contain proteins and are merely layers of cytoplasm separating vacuoles from one another (Fig. 51, *v*).

The basidia taper slightly at both ends and become protuberant. At first it seemed that the basidia were dimorphic, *i.e.* that some were longer and more protuberant and others shorter and less protuberant; but this supposition turned out to be an error. Basidia which have spores about to be discharged are all equally protuberant; but young basidia begin to develop sterigmata and spores before their bodies have attained full length (Fig. 51). This I have not yet noticed in any other species. The result is that

basidia with young spores are shorter and less protuberant than basidia with mature spores. During the development of the spores, the basidium-body gradually elongates and so raises the spores into a higher and higher position as they become more and more mature.

FIG. 51.—*Pluteus cervinus*. Transverse section through the hymenium, about the third day of the spore-discharge period, to show the relations of the cystidia with the other lamellar cells: *h*, the hymenium; *s*, the subhymenium; *t*, the trama. The two cystidia, *a* and *b*, spring from tramal cells and each bears an apical crown of more or less recurved prongs. Their protoplasm encloses large vacuoles, *v*, and the parts which project into the interlamellar space have very thick walls, *w*. Other hymenial elements: *c*, collapsed basidia which have discharged their spores; *d*, basidia bearing ripe or almost ripe spores, with basidial bodies of full length; *e*, younger basidia with sterigmata or very young spores, with basidial bodies still growing at their ends and therefore not yet fully protuberant; *p*, paraphyses. Magnification, 733.

The end of the basidium, just beneath the sterigmata, becomes more slender than the main shaft during the final lengthening of the basidium-body and appears to be responsible for the elongation in question.

The paraphyses (Fig. 51, *p*) are small somewhat oval cells, which gradually swell in size as the hymenium becomes older and as more and more basidia discharge their spores and collapse. They never

develop slender apices comparable with those of the basidia, and their protoplasmic contents soon become reduced to a very thin layer lining the wall and enclosing one large clear vacuole. They do not form a compact system, but exist in the form of loosely connected strings like those already described for *Panaeolus campanulatus* and *Stropharia semiglobata*.

The discharge of spores from the hymenium of two gills into an interlamellar space during the spore-discharge period is illustrated in Fig. 49 at E (p. 106). The arrows indicate the trajectories described by some of the projectiles when shot away violently from their sterigmata. Each spore is projected more or less perpendicularly outwards from the hymenium to a distance of about 0·1 mm. and then, with diminishing horizontal velocity, it turns rather sharply downwards, afterwards falling under the influence of gravity alone with a steady terminal velocity of about 0·67 mm. per second.[1] The spores were found to have an average length of only 5·95 μ and an average width of only 4·57 μ,[2] and there can be no doubt that they fall at the relatively slow speed of 0·67 mm. per second owing to their very small size. The gills shown at D in Fig. 47 (p. 103) are about 1 cm. deep. If a spore were liberated at the top of one of these gills in still air, a simple calculation shows that it would take about 15 seconds to fall completely down the interlamellar space before it could emerge from beneath the pileus and escape into the outer air.

When a spore of *Pluteus cervinus* is about to be shot away from its sterigma, the usual drop of fluid is excreted at the hilum, as is shown on the right of Fig. 49, E, near the letter *h*. A spore, when shot away, is always accompanied by its drop, and two of the falling spores in E are shown with drops attached. However, the drops, owing to their extremely small size, evaporate very rapidly indeed. Hence most of the falling spores in E are represented as no longer accompanied by their drops. The spores themselves also dry up very quickly and, in consequence, during their fall, become boat-shaped in form. Two boat-shaped spores are to be seen in E in the middle of the figure.

Finally, we may ask: what is the function of the cystidia?

[1] These *Researches*, vol. i, 1909, pp. 175. [2] *Ibid.*, p. 162.

There is perhaps no very obvious answer to this question. The large number of cystidia and the way in which they cross the interlamellar spaces of as yet unexpanded fruit-bodies suggest that they serve as organs for maintaining the existence of the interlamellar spaces and thus for preventing the opposing surfaces of adjacent gills from rubbing on one another during the development of the first spores. If this function is the true one, then we have a physiological similarity with what obtains in *Coprinus atramentarius* and certain other Coprini.[1] The cystidia in the young unexpanded fruit-body often completely cross the interlamellar spaces, but the hooks do not become entangled in the hymenium of the opposing gill (*cf.* Fig. 49, D, p. 106). The hooks on a cystidium are always produced apically and are turned outwards: thus they increase the surface of the end of the shaft. Possibly, therefore, the shaft is thereby better fitted to push against the opposing gill and thus to maintain the existence of the interlamellar space. There seems to be no evidence that the sharp hooks are used to penetrate anything. Another fact which supports the idea that the cystidia are interlamellar space-makers is that the wall of that part of each cystidium which projects across an interlamellar space is much thickened and thereby greatly strengthened mechanically (*cf.* Fig. 51, p. 108).

Stahl found that the acicular raphides which occur in bundles in the leaves of many Monocotyledons, *e.g. Arum maculatum*, render the leaves inedible to slugs, owing to the fact that they irritate the mucous membrane of the mouth in a mechanical manner.[2] It struck me that possibly the hooked cystidia might have a similar protective function. I therefore collected slugs of three species from the garden and, after starving them for a few hours, offered them pieces of the pileus of *Pluteus cervinus* to eat. One of the slugs (*Limax maximus ?*) ate freely of the gills, and must have devoured thousands of cystidia. Dr. W. T. Elliott found that, under laboratory conditions, the fruit-bodies of *Pluteus cervinus* are readily eaten by *Limax maximus* and *L. cinereo-niger*, and slightly eaten by *Arion ater*.[3] The hooked cystidia do not therefore appear to be

[1] *Vide infra.*

[2] E. Stahl, *Pflanzen und Schnecken*, Jena, 1888, p. 85.

[3] W. T. Elliott, *in litt.*

protective in function, and the supposed analogy with raphides is not supported by experiment.

Nolanea pascua.—This species commonly comes up on lawns and in grassy fields in England and, like *Pluteus cervinus*, has pink spores. Its gills conform in general shape and orientation to the rule for the Aequihymeniiferae, *i.e.* the gills are wedge-shaped in cross-section and look vertically downwards to the earth. However, a gill is often locally irregular in that here and there, particularly in the upper half, it bulges outwards. Each such bulge may prevent the free escape of spores discharged above it by offering mechanical hindrance to their fall. The spores then collect on the upper side of such a bulge, with the result that the gill becomes powdered locally. Almost every fruit-body of *Nolanea pascua* comes to have some of its gills powdered in this way.

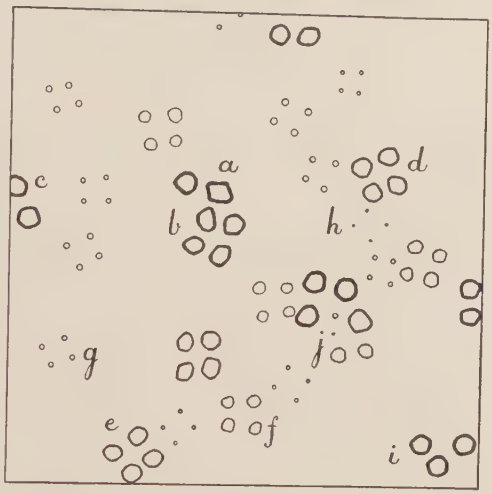


FIG. 52.—*Nolanea pascua.* Surface view of hymenium in the middle of the spore-discharge period, showing spores: *a*, a basidium which has just shot away two of its spores; *b* and *c*, nearly ripe spores; *d* and *e*, spores which have just attained full size; *f*, half-grown spores; *g*, very rudimentary spores; *h*, ends of sterigmata about to develop spores; *i*, a basidium with three spores only; *j*, two four-spored basidia, the upper with three well-grown spores and one aborted, the lower with one well-grown and three aborted (one very small). Magnification, 330.

The spores, when mature, remotely resemble crystals, for they have several more or less flattened sides and straight edges. Their growth was observed by the method described in Volume II.[1] Each takes about 2·5 hours to attain full size, after which there is an interval of about 1·75 hours before discharge takes place. The total amount of time required for the development and discharge of a single spore is therefore about 4·25 hours.

[1] *Researches on Fungi*, vol. ii, 1922, pp. 43–45.

A tiny drop of water is excreted at the hilum of each spore just before discharge, and the drop is carried with the spore. The means employed for proving that the drop is carried with the spore was fully described in Volume II.[1]

The spore-bearing basidia are scattered over the hymenium in the manner characteristic for the Armillaria Sub-type (Fig. 52). The older basidia with nearly ripe spores are well separated from one another and younger spore-bearing basidia come up between them in a scattered manner. There is no compact grouping of spore-bearing basidia of like age such as we find in *Panaeolus campanulatus*.

Concluding Remarks.—The Armillaria Sub-type, in which spore-bearing basidia of about the same degree of maturity are set distantly apart with younger spore-bearing basidia coming up between them, includes a considerable number of species belonging to the Leucosporae and the Rhodosporae.

Associated with the loose arrangement of the hymenium in the Armillaria Sub-type there is, on the whole, a relatively rapid development of the individual spores as compared with the Panaeolus Sub-type. This is shown in the table on p. 113.[2]

Finally, one may ask : which is the more primitive arrangement of the hymenium, the loose one of the Armillaria Sub-type or the compact one of the Panaeolus Sub-type ? The former seems to me to be the more primitive. One may suppose that there is more detailed co-ordination between the elements of the hymenium in securing the compact arrangement and the successive development of a series of generations of basidia in a Panaeolus, than is displayed in the loose arrangement of the hymenium exhibited, say, in *Collybia velutipes*. It seems probable that the Porphyrosporae and the Melanosporae, with their highly pigmented spores, have been evolved from the Leucosporae, with their colourless spores, and that, during the transition, the compact type of hymenium was developed from the loose type. Also the hymenium of the Armillaria Sub-type may be regarded as more primitive than that of the Psathyrella Sub-type, in which the basidia are specialised to the extent of being

[1] *Researches on Fungi*, vol. ii, 1922, pp. 15–17, also Fig. 7.

[2] The data are taken from vol. ii, 1922, p. 44.

polymorphic and the paraphyses are enlarged and organised so as to form a continuous system. The Bolbitius Sub-type, of those so far discussed, stands nearest to the Armillaria Sub-type, for its spore-bearing basidia are more or less loosely spaced or have a

Average Time taken for the Development and Ripening of an Individual Spore from the Moment the Spore appears on the Sterigma as a Tiny Rudiment until the Moment of Discharge.

		Hours	Minutes
Armillaria Sub-type	*Leucosporae*		
	Collybia velutipes	0	47
	Collybia fusipes	1	3
	Marasmius oreades	1	5
	Armillaria mellea	1	30
	Collybia radicata	1	30
	Russula cyanoxantha	5	10
	Rhodosporae		
	Pluteus cervinus	3	25
	Nolanea pascua	4	15
Panaeolus Sub-type	*Porphyrosporae*		
	Stropharia semiglobata	5	40
	Psalliota campestris	8	0
	Melanosporae		
	Panaeolus campanulatus	7	30
	Anellaria separata	10	30

scattered arrangement; but, in a Bolbitius, the basidia are restricted in number in correlation with the ephemeral nature of the fruit-body, and the paraphyses are relatively very large and form a continuous system of cells in which the basidia are embedded. I am inclined to believe that the Armillaria Sub-type has been the parent of all the other Sub-types of the Aequi-hymeniiferae, including the Inocybe Sub-type about to be described.

CHAPTER V

THE INOCYBE SUB-TYPE AND ITS INTERMEDIATE POSITION

General Remarks—Characters of the Inocybe Sub-type

General Remarks. — The Panaeolus, Psathyrella, Bolbitius, and Armillaria Sub-types, when typically developed, can be readily distinguished from one another with the help of the microscope. The Inocybe Sub-type, with which this Chapter is concerned, stands in a position intermediate between the Panaeolus and the Armillaria Sub-types and serves, more or less, to bridge over the gap between them. This is the most unsatisfactory Sub-type with which we have to deal, and in it have been placed those fungi in which (1) the mottling of the hymenium is very feebly developed, so that one cannot detect it with the naked eye or even in any very clear manner with the microscope, but in which, nevertheless, (2) the basidia of like age are not widely scattered over the hymenium but come up in tiny irregular groups or strings. We seem to have presented to us fungi which are passing from the Armillaria to the Panaeolus Sub-type, or *vice versa*, and in which the basidia of like age come up not isolated from one another but in small associations.

The species of Hymenomycetes which I have been unable to place in either the Panaeolus or the Armillaria Sub-type, but which appear to occupy an intermediate position and have therefore been included in the Inocybe Sub-type, are as follows :

Inocybe asterospora	Psathyra corrugis
Galera tenera (Fig. 53)	Russula emetica

Characters of the Inocybe Sub-type.—The Inocybe Sub-type of fruit-body possesses all the characters already described for the Aequi-hymeniiferous Type : the gills are wedge-shaped in cross-

section and positively geotropic, the hymenium looks downwards to the earth, and every part of the hymenium produces and liberates spores during the whole period of spore-discharge.

The special characters of the Inocybe Sub-type resemble those of the Panaeolus and the Armillaria Sub-types, except for the intermediate structure of the hymenium, and may be stated as follows :

(1) The whole structure of the fruit-body is relatively stout and

FIG. 53.—*Galera tenera*. Three fruit-bodies which grew amid grass. Collected at Ottawa by W. S. Odell. Photographed by the Photographic Division of the Geological Survey of Canada. Natural size.

suited to a persistence of the opened pileus for several days. In this respect, therefore, the Inocybe Sub-type resembles the Panaeolus and Armillaria Sub-types, but differs from the Psathyrella and the Bolbitius Sub-types.

(2) The spore-discharge period is relatively long : its duration is for several days. Here again, we have a contrast with the Psathyrella and the Bolbitius Sub-types but an agreement with the Panaeolus and the Armillaria Sub-types.

(3) In species with pigmented spores, the gills are not strongly

mottled and therefore differ from those of the Panaeolus Sub-type. If there is any mottling present, it cannot be definitely observed with the naked eye and only somewhat vaguely with the microscope.

(4) On any small area of the hymenium the basidia here and there are associated into little strings or groups of like, or almost like, age ; so that one can recognise an arrangement of the basidia which, when the spores are pigmented, results in what may be called *incipient mottling*. Owing to this arrangement, the fungi of the Inocybe Sub-type appear to be closely connected with those of the Panaeolus Sub-type.

(5) The basidia are monomorphic, *i.e.* equally protuberant. In this respect the Inocybe Sub-type agrees with the Panaeolus, the Bolbitius and the Armillaria Sub-types, but differs from the Psathyrella Sub-type as well as from all the Coprinus Sub-types.

(6) There is no lateral crowding of the basidia with overlap of spores such as is found in the Psathyrella Sub-type and all the Coprinus Sub-types, but adjacent basidia bearing spores are set rather closely together. The hymenium is therefore somewhat compact as regards the spore-bearing basidia, and in this respect it agrees rather with the Panaeolus than with the Armillaria Sub-type.

(7) The paraphyses are like those of the Panaeolus and the Armillaria Sub-types, *i.e.* are small and not joined laterally into a definite hymenial pavement such as we find in the Psathyrella and the Bolbitius Sub-types.

The intermediate position of the Inocybe Sub-type in respect to the Panaeolus and the Armillaria Sub-types will have been sufficiently elucidated by the above remarks. I can easily conceive that one investigator might consider a particular fungus as belonging to the Inocybe Sub-type, while another might consider it as belonging to the Panaeolus Sub-type. It is difficult to draw an exact line clearly separating these two Sub-types. Similarly, it might happen that one investigator would place a particular fungus in the Armillaria Sub-type, and another in the Inocybe Sub-type. When there is uncertainty, it would be best to place the doubtful fungus in the Inocybe Sub-type, for this has been established to include intermediate forms.

Nature revels in variety and continuity, and the existence of such an intermediate Sub-type as the Inocybe affords further evidence of this fact. Doubtless, if a particular area of the hymenial surface of any Agaric belonging to this Sub-type were to be watched with the microscope continuously for several hours or days, as has been done for *Panaeolus campanulatus*,[1] a much more complete understanding of the organisation of the hymenium might be obtained than that which has here been presented.

[1] These *Researches*, vol. ii, 1922, pp. 260–264.

CHAPTER VI

THE INAEQUI-HYMENIIFERAE

The Establishment of the Genus Coprinus—Chief Characters—Other Characters—Previous Investigations—Critical Remarks on the Generic Position of Certain Agaricineae—*Psathyra urticaecola*—*Coprinus plicatilis*—Falck's Theory of Radiosensitivity in Coprinus

The Establishment of the Genus Coprinus. The Inaequi-hymeniiferous or Coprinus Type of fruit-body is more highly specialised than the Aequi-hymeniiferous or Non-Coprinus Type from which in all probability it has been evolved. Its most striking characteristic is the deliquescence or, as I prefer to call it, the *auto-digestion* of the gills during the discharge of the spores. This phenomenon early attracted the attention of systematic mycologists. In 1801, Persoon, in his *Synopsis Fungorum*, gathered together all the lamellated fungi exhibiting deliquescence into a section of the genus Agaricus and called this section *Coprinus* in reference to the fact that many of the species come up on the dung of herbivorous animals. Fries, in his *Systema Mycologicum* of 1821, retained this arrangement, so that for several decades such a fungus as *Coprinus comatus* was known to mycologists as *Agaricus* (*Coprinus*) *comatus*. However, Fries, with the publication of his *Epicrisis Systematis Mycologici*, 1836–1838, raised Persoon's section Coprinus to generic rank, a status which has been rightly maintained by all modern systematists. It thus happens that all the fungi having fruit-bodies of the Inaequi-hymeniiferous or Coprinus Type are included in the single genus Coprinus.

Chief Characters.—The chief points in connection with the mechanism for the production and liberation of the spores for both the Aequi-hymeniiferous and the Inaequi-hymeniiferous Types

were given in Volume II, Chapter IX. Those for the Inaequi-hymeniiferous or Coprinus Type may be summarised as follows :

(1) The gills are very thin.
(2) The gills are parallel-sided or subparallel-sided.
(3) The gills are not positively geotropic.
(4) Usually the hymenium on one side of the gill at maturity looks slightly downwards and that on the other side slightly upwards.
(5) The spores ripen in succession from below upwards on each gill.
(6) The spores are discharged in succession from below upwards on each gill.
(7) Autodigestion proceeds from below upwards on each gill and removes those parts of the gills which have become spore-free and which, if they continued in existence, would become mechanical hindrances to the fall of the remaining spores.

All the above facts are correlated with one another. *In the genus Coprinus the ripening and discharge of the spores from below upwards on each gill, and the autodigestion from below upwards on each gill, are special arrangements which permit of successful spore-discharge from parallel-sided non-geotropic gills.* This conclusion is drawn from the facts which will be brought forward in giving an account of various Coprini in subsequent Chapters, and it is set forth here so that the reader may have a guide from the first for the understanding of the various Sub-types of the Coprinus Type which are about to be described.

The chief points in connection with the mechanism for the production and liberation of spores in the Aequi-hymeniiferae or Non-Coprinus Agaricineae, which contrast with those already enumerated for the Inaequi-hymeniiferae, may be summarised as follows :

(1) The gills in a few species are very thin but in most species are relatively thick.
(2) The gills are not parallel-sided, but are more or less acutely wedge-shaped in cross-section.
(3) The gills are positively geotropic.

(4) Every part of the hymenium on any gill in a normally oriented fruit-body looks more or less downwards.

(5) The spores do not ripen in succession from below upwards on each gill but more or less uniformly all over the gill-surfaces.

(6) The spores are not discharged in succession from below upwards on each gill but more or less uniformly all over the gill-surfaces.

(7) Autodigestion does not proceed from below upwards on each gill or involve any part of a gill during the period of spore-discharge.

With the help of the seven criteria enumerated above one can readily determine whether or not any particular fungus should be included in the genus Coprinus, *i.e.* in the Inaequi-hymeniiferae.

Other Characters.—With the chief characters of the Coprinus Type are associated and correlated others of less fundamental importance but of no little interest, which are concerned with the colour of the gills, the nature of the elements making up the hymenium, the sub-zones of the zones of spore-development and spore-discharge, the general structure of the pileus-flesh and of the stipe, the length of the spore-discharge period, and the ephemeral nature of the Coprinus Type taken as a whole. Of these we shall now treat.

(1) The gills are neither mottled nor perfectly even in colour texture, for during development they blacken from below upwards. This blackening of the gills from below upwards is a well-known field character for the larger Coprini, such as *Coprinus comatus* and *C. atramentarius*, but it also occurs in the smaller Coprini, such as *Coprinus ephemerus* and *C. stercorarius*. It is due to the fact that the spores, whilst ripening, develop a deep brown pigment in their walls and also to the fact that the spores on a single gill ripen in succession from below upwards.

(2) The number of generations of basidia, as a rule, is limited to two. These two generations overlap so that the younger one is only slightly behind the older one in beginning and ending its development. Finally the spores of neighbouring basidia of both generations come to be ripe and ready for discharge at approxi-

mately one and the same time. There is nothing exactly corresponding to this among the Aequi-hymeniiferous Sub-types. Thus, in the Panaeolus Sub-type, the generations of basidia on any one small area of the hymenium are about ten in number, and one generation of basidia does not begin to develop its spores until the previous one has discharged its spores. The nearest parallel among the Aequi-hymeniiferae is to be found in the Psathyrella Sub-type, for there the number of generations of basidia is limited to four and there is overlapping in the development of the successive generations. However, the spores on adjacent basidia of the four generations do not all come to be ripe and ready for discharge at approximately the same time, but ripen and are discharged during four successive, although somewhat overlapping, periods of time.

(3) The basidia, as a rule, are dimorphic. They are never monomorphic, although they are occasionally trimorphic and very rarely tetramorphic. In the hymenium of most species of Coprinus, where the basidia are dimorphic, the first-generation basidia are much longer than those of the second generation. The occasional polymorphism of the basidia, which occurs in a few species only, finds its parallel in the tetramorphism of the basidia of the Psathyrella Sub-type of the Aequi-hymeniiferae. Basidial dimorphism, so far as I know at present, is confined to the Coprini. If, therefore, on examining the hymenium of a strange fungus in face view with the microscope, one perceives that the spores are black and the basidia dimorphic, one has very strong *prima facie* evidence that the fungus is a Coprinus.

(4) The basidia of the two generations are interspersed among one another throughout the hymenium and are crowded together, so that frequently spores of the first-generation basidia stand in part vertically above spores of the second-generation basidia. This phenomenon is not limited to the Coprini, for, as we have seen, it occurs also in the Psathyrella Sub-type. We made its acquaintance in connection with the descriptions of *Psathyrella disseminata* and *Lepiota cepaestipes.*

(5) While, broadly speaking, the basidia develop their spores in succession from below upwards on each gill and shed their spores in succession from below upwards on each gill, or while, in other

words, a zone of spore-development passes from the bottom to the top of each gill, yet in these zones the basidia of the first generation always take a slight lead in the times of the production and discharge of their spores. In consequence of this, the broad zones of spore-development and spore-discharge are each composed of two sub-zones, a higher one belonging to the first-generation basidia and a lower one belonging to the second-generation basidia.

(6) Paraphyses are normal constituents of the hymenium. They are elements which are destined to remain sterile until their destruction by autodigestion. They are very large and are united so as to form a pavement through which the basidia protrude. They not only support the basidia mechanically, but act as space-makers so that adjacent basidia are separated from one another by a distance just sufficient to prevent any jostling during spore-development and spore-discharge. A similar system of paraphyses occurs in the Psathyrella and Bolbitius Sub-types among the Aequi-hymeniiferae.

(7) The pileus-flesh is very thin. Its amount is very small in proportion to the large area of hymenial surface. This is particularly noticeable when one compares large Coprini, *e.g. Coprinus comatus* and *C. atramentarius*, with large Aequi-hymeniiferae, *e.g. Lepiota procera*, *Amanita rubescens*, and species of Lactarius, Russula, etc. In the smaller species of Coprinus, the very thin flesh usually splits into radial ribbons as the pileus opens, and the lines of cleavage are usually continued down the middle planes of the gills so that the pilei open out like parasols. We find a similar condition in a few Aequi-hymeniiferae, among which may be mentioned certain species of Bolbitius and *Psathyrella disseminata*. This last species, however, does not expand beyond the campanulate stage. The thin flesh of the Coprini in general is correlated with the peculiar mode of the production and liberation of the spores. This fact is best realised from a study of the larger Coprini, *e.g. Coprinus comatus* and *C. atramentarius*.

(8) The stipe is hollow and its substance usually rather brittle. In no species of Coprinus is the stipe solid. In the Aequi-hymeniiferae, on the other hand, some species have solid stipes and some hollow. The hollow space in the stipe of a Coprinus is usually large relatively to the thickness of the stipe-wall. The structure of the

stipe is suited only for a very brief existence of the fruit-body as a whole.

(9) The discharge of the spores is, as a rule, very rapidly accomplished and, as soon as it is ended, the fruit-body collapses. Spore-discharge is completed in some small species, *e.g. Coprinus curtus*, in the course of about 2 hours ;[1] in larger species, *e.g. Coprinus niveus* and *C. stercorarius*, in less than 12 hours ; and in the largest species, *e.g. Coprinus comatus* and *C. atramentarius*, in less than 48 hours. The length of the spore-discharge period in the Coprini chiefly depends on the vertical distance on the gills up which the zone of spore-discharge has to pass. In very small species it may have to go a distance of only about 1 mm., in medium-sized species it may require to travel several mm. or 1 cm., while in the largest species it may have to move upwards for several cm. From what has just been said it is evident that, on the whole, the Coprinus Type of fruit-body has but a very ephemeral existence. In this respect it resembles the Bolbitius and the Psathyrella Sub-types among the Aequi-hymeniiferae.

A full discussion of the organisation of the Coprinus Type of fruit-body will be given after the development and structure of particular Coprini have been described in detail.

Previous Investigations.—It is not improbable that the first reference to Coprini in botanical literature is to be found in the statement by Theophrastus, *circa* 300 B.C., that the fungi which grow on dung have no bad smell.[2] In the year 1727 Vaillant gave a careful description and some excellent copper-plate illustrations of *Coprinus atramentarius*.[3] In the course of his description he states that " the gills are very closely set one against the other, attain a width of six or seven lines, and are of a dirty white colour which gradually turns black as the cap opens " ; and, in one of his illustrations which represents a vertical section through a fruit-body, he depicts the gills in the act of blackening from below upwards ; but he appears to have overlooked the deliquescence of

[1] These *Researches*, vol. ii, 1922, p. 99.

[2] Theophrastus, *De Odoribus*, Frag. IV, 1, §3, ed. Teubner.

[3] S. Vaillant, *Botanicon Parisiense*, Leide et Amsterdam, 1727, p. 73, Pl. XII, figs. 11 and 12.

the pileus and he makes no reference to the spores, the existence of which upon the gills he probably did not even suspect.

The spores of the Coprini were discovered in 1729 by the Florentine botanist Micheli who observed them upon the gills with the microscope.[1] In his illustrations he shows them dotted over the surface of the gills, accompanied by numerous cystidia. He thought that the spores were seeds, like those of the higher plants, and he suggested that the cystidia act as mechanical stays for holding the gills apart and thus permitting of the free fall of the spores through the interlamellar spaces.[2] This was a shrewd guess, which has been justified by my own researches for certain Coprini, notably *Coprinus atramentarius*.[3] Micheli's illustrations prove that he was acquainted with several species of Coprinus and that among them were *Coprinus macrorhizus* and *C. sterquilinus*.[4] However, the gradual melting down of the pilei during the discharge of the spores appears to have escaped his notice.

O. F. Müller, in 1780, described *Coprinus comatus* in the *Flora Danica*. He gave not only some excellent life-size illustrations showing the fruit-bodies in various stages of development including the deliquescence of the gills and the production of inky drops from the revolute pileus-margin, but also a sketch of a surface view of the hymenium as seen with the microscope. In this sketch he shows the spores in groups of four, the basidia beneath them, the sterile paraphyses and, at the edge of the drawing, the sterigmata supporting the spores.[5] No essential point in the structure of the hymenium, as seen in surface view, was missed. However, Müller does not seem to have understood the interest of his drawing, for, strangely enough, he made no comment upon it : he simply represented what he had seen with the microscope and thereby lost the splendid opportunity of being the first to give an account of the general structure of the hymenium of the Agaricineae.

[1] A. H. R. Buller, "Micheli and the Discovery of Reproduction in Fungi," Presidential Address to Section IV, Roy. Soc. of Canada, *Transactions*, vol. ix, 1915. In this publication I have reproduced in four Plates Micheli's illustrations of spores and spore-bearing organs.

[2] Micheli, *Nova Plantarum Genera*, Florentiae, 1729, p. 133, Tab. 73.

[3] *Vide infra*, Chap. IX.

[4] Micheli, *loc. cit.*, Tab. 80, Figs. 2 and 3.

[5] O. F. Müller, *Florae Danicae Icones*, Fasc. XIV, 1780, Tab. 834.

Bulliard, in 1791, gave a good illustration of the cystidia on the gills of a species of Coprinus ; but, instead of following Micheli and attributing to the cystidia a mechanical function, he regarded these structures as male organs producing a seminal fluid which is employed to fertilise the so-called seeds (spores).[1] This spermatic theory of the function of the cystidia of Coprini found adherents in Corda, 1834 and 1839, and Klotzsch, 1838, and was only disproved through the careful observations of Hoffmann in 1856.[2] It was even upheld by Worthington Smith as late as 1875.[3]

After Hedwig had shown in 1788 that the fleshy Discomycetes have their spores contained in thecae (asci), the erroneous belief grew up that what we now call the Hymenomycetes also have their spores enclosed in thecae. Persoon, on this assumption, included both Discomycetes and Hymenomycetes in his Sixth Order *Hymenothecium*.[4] Link, in 1809, made matters worse by publishing illustrations of the hymenium of a Coprinus in which he represented the basidia as thecae, each theca containing four rows of spores.[5] He represented the basidia of several other Hymenomycetes as containing a single row of spores.[6] Link's mistaken illustration of the hymenium of Coprinus was copied into Nees von Esenbeck's text-book of Fungi and served to lead everybody astray.[7] Other botanists followed Link like so many sheep, and among those who succeeded in discovering spore-containing asci in Hymenomycetes were : Ditmar in 1813, Nees von Esenbeck in 1817, Sprengel in 1817, Ehrenberg in 1821, Greville in 1825–1828, Desmazières in 1828, Fries in 1830, Klotzsch in 1833, and Corda whose illustrations of thecae in Hymenomycetes are to be found throughout the pages

[1] Bulliard, *Histoire des Champignons de la France*, Paris, 1791, pp. 32–33, etc.

[2] Hoffmann, "Die Pollinarien und Spermatien von Agaricus," *Bot. Zeit.*, Bd. XIV, pp. 136–148.

[3] W. G. Smith, *Reproduction in Coprinus radiatus*, Grevillea, vol. iv, 1875–1878, pp. 53–56.

[4] Persoon, "Neuer Versuch einer systematischen Eintheilung der Schwämme," *Romer's Neues Mag. f. d. Bot.*, Bd. I, 1794, pp. 63–128.

[5] Link, "Observationes in Ordines plantarum naturales, etc.," *Mag. d. Ges. naturf. Freunde*, Berlin, Bd. III, pp. 35–37, Tab. II.

[6] Link, "Nova plantarum genera, etc.," *Schrader's Neues Journ. f. d. Bot.*, Jg. III, 1809.

[7] C. G. Nees von Esenbeck, *Das System der Pilze und Schwämme*, Würzburg, 1817, Tab. XXV.

of Krombholz and of Sturm's *Deutschlands Flora*. Finally the bubble was burst between the years 1836 and 1842 through the work of Ascherson, Léveillé,[1] and Berkeley,[2] supplemented by that of Phœbus and the later work of Corda : the supposed asci melted into thin air and were replaced by typical basidia with external spores such as everybody recognises at the present day. The first illustrations of a cross-section and a surface section of the hymenium of a Coprinus showing basidia and paraphyses correctly drawn were published by Corda in 1837.[3]

A considerable advance in our knowledge of the Coprini was made by Brefeld who in the year 1877 gave a masterly account of the origin and development of *Coprinus stercorarius*.[4] Brefeld showed that a fruit-body arises from a single cell of the mycelium, and not from an oospore or zygospore as had been previously supposed. He raised the fungus in pure cultures and followed its growth step by step from spore to spore. He showed that the sclerotium is a resting body, laden with food materials and destined to produce fruit-bodies under favourable conditions. He described the histology of the stipe, the pileus-flesh, and the gills, and illustrated his remarks with a series of excellent drawings. As shown by his cross-sections of a gill, he clearly perceived the true structure of the basidia, the paraphyses, and the cystidia ; and he discussed the function of each. He rightly maintained that the spores are shot away with violence from the ends of the sterigmata, that the paraphyses are sterile elements which play an important part in the expansion of the gills and thereby of the pileus, and that the cystidia act as stays which prevent the gills from pressing upon one another and thus bring into existence the spaces required for the development of the spores. Notwithstanding all Brefeld's care and his desire for complete analysis, certain important points in the organisation of the Coprinus type of fruit-body escaped his notice.

[1] Léveillé, "Recherches sur l'Hyménium des Champignons," *Ann. sci. nat.*, 2 sér., VIII, 1837.

[2] Berkeley, "On the Fructification of the Pileate and Clavate Tribes of Hymenomycetous Fungi," *Ann. Nat. Hist.*, London, I, 1838, pp. 81–101.

[3] Corda, *Icones Fungorum*, Tomus I, Prague, 1837, Bd. I, Tab. VII.

[4] O. Brefeld, *Untersuchungen über Schimmelpilze*, Leipzig, Heft III, 1877, pp. 1–97.

Among these were : the dimorphism of the basidia and the associated crowding of the basidia on the hymenium, the fate of the cystidia, the fact that the spores ripen in succession on each gill from below upwards, and the significance of the phenomenon of deliquescence.[1]

Massee [2] in 1896 gave a revision of the genus Coprinus in which he enumerated 169 species and discussed their general morphology, their distribution, their habitat, and their classification. However, he was unable to add anything to our knowledge of the mechanism of the fruit-bodies as organs for the production and liberation of the spores. He even overlooked the fact that the spores are developed and discharged from below upwards on the gills, and he entirely misinterpreted the meaning of the drops produced by the deliquescence of the gills and pileus-flesh. Massee says : "The species of Coprinus differ from the remainder of the Agaricineae in one important biological feature,—the deliquescence of the gills at maturity into a liquid which drips to the ground, carrying the mature spores along with it. This primitive and relatively imperfect mode of spore-dissemination, as compared with the minute, dry, wind-borne spores of the remainder of the Agaricineae, combined with other evidence to be noted later on, indicates that in the genus Coprinus we have, in reality, the remnant of a primitive group of Fungi from which have descended the entire modern group of Agaricineae having wind-borne spores." [3] I showed, in Volume I of these *Researches*, that the spores of the Coprini *are* wind-borne and that, consequently, Massee was in error in supposing they are not. When the wind-borne character of the spores is admitted, Massee's chief argument for regarding the genus Coprinus as being the remnant of a group from which all the other Agaricineae have been evolved is destroyed. In the first Volume of these *Researches* I gave reasons

[1] Brefeld's errors of observation were few. Those which I have noticed are : the details of the discharge of the spores from the sterigmata (*cf.* these *Researches*, vol. i, pp. 134–135 ; vol. ii, p. 4) ; the representation of all the basidia in a cross-section of the hymenium as being long and much protuberant instead of as being long and short (*Untersuchungen, loc. cit.*, Pl. IV, Figs. 11 and 12), and the statement that the basidia all begin to develop spores, etc., at one and the same time throughout the pileus (*Untersuchungen, loc. cit.*, p. 56).

[2] G. Massee, "A Revision of the Genus Coprinus," *Annals of Botany*, vol. x, 1896, pp. 123–184 and two Plates.

[3] *Ibid.*, pp. 129–130.

for believing that the Coprinus Type of fruit-body has been derived by specialisation from the Non-Coprinus Type and not *vice versa*.[1]

Lange,[2] in 1915, published a revision of the Danish Coprini in which he enumerated 32 species and gave an original description of each, including certain microscopic characters. However, he did not discuss the mechanism of the fruit-bodies for the production and liberation of the spores or seek to explain the melting away of the gills. He was evidently unacquainted with my studies published in the years 1909, 1910, 1912, and 1914, in which I indicated the true limits of the genus Coprinus; for, were it otherwise, I do not think he would have brought forward the view that Bolbitius can be regarded as a sub-genus of Coprinus or have followed Quélet in removing *Psathyrella disseminata* and *P. impatiens* from the genus Psathyrella, where Fries had placed them, and in including them in Coprinus.

The biological significance of the phenomenon of deliquescence remained entirely unknown until the publication of the first volume of these *Researches* in the year 1909. In that work I described in detail the mode of the production and liberation of spores in *Coprinus comatus* [3] and pointed out that, in this species, as in Coprini in general: (1) the spores on each gill ripen in succession from below upwards, (2) the spores on each gill are discharged in succession from below upwards, and (3) deliquescence is a process of autodigestion, which on each gill proceeds from below upwards and destroys those parts of the gill which have already shed their spores and which, if they continued in existence, would hinder the fall of the remaining spores. It thus became clear that autodigestion has a perfectly definite and useful function to fulfil in connection with the liberation of the spores.

Since 1909 my studies of the organisation of Coprinus fruit-bodies have been continued, and a number of further details of

[1] A. H. R. Buller, *Researches on Fungi*, vol. i, 1909, pp. 214–215. Presumably it was on account of his belief in the ancestral position of the genus Coprinus that Massee placed the Melanosporae before the other families of Agaricineae in his *British Fungus-Flora*.

[2] Jakob E. Lange, "Studies in the Agarics of Denmark," *Dansk Botanisk Arkiv*, Bd. II, Copenhagen, 1915.

[3] A. H. R. Buller, *Researches on Fungi*, vol. i, 1909, chap. xix, pp. 196–215.

structure and function have been brought to light. In 1910, I published an account of *Coprinus atramentarius* in which were described the function and fate of the large cystidia which occur in this species.[1] It was shown that the mechanism for the production and liberation of spores in *C. atramentarius* is essentially the same as in *C. comatus*, but that the two species differ in the manner in which adjacent gills are prevented from coming into contact during the ripening and discharge of the spores. In *Coprinus comatus* the surfaces of adjacent gills are kept apart owing to the fact that the gill-edges are swollen so that each forms a flange, whilst in *C. atramentarius* the surfaces are kept apart by the presence of numerous cystidia which stretch across the interlamellar spaces and act as stays or distance-pieces. In *Coprinus atramentarius* the gills are very broad and yet extremely thin : consequently they are lacking in rigidity. The presence of the cystidia is absolutely necessary in this species to prevent adjacent gills from touching one another, and is therefore an essential factor in the successful production and liberation of the spores. I called attention to the fact that the gills of *Coprinus atramentarius* are parallel-sided and stated the important conclusion that, in the Coprini, the ripening and discharge of the spores from below upwards on each gill, and the gradual autodigestion of the spore-free portions of the gills from below upwards, are to be regarded as adaptations which permit of successful spore-liberation from parallel-sided gills.

In a further paper, in 1912, I pointed out that in the Coprini the gills, unlike those of most Agaricineae (*Psalliota campestris*, etc.), do not react to the stimulus of gravity, *i.e.* they are *ageotropic*, and also that the basidia are *dimorphic*.[2] The basidia in the hymenium of most Coprini (*Coprinus comatus*, *C. atramentarius*, *C. stercorarius*, *C. ephemerus*, etc.) are of two kinds, long and short. The long and short basidia are interspersed among one another and among the paraphyses, so as to form a beautiful mosaic-work. It was shown

[1] A. H. R. Buller, "The Function and Fate of the Cystidia of *Coprinus atramentarius* together with some General Remarks on Coprinus Fruit-bodies," *Annals of Botany*, vol. xxiv, 1910, pp. 613–629.

[2] A. H. R. Buller, "The Production and Liberation of Spores in the Genus Coprinus," *Transactions of the British Mycological Society* for 1911, vol. iii, 1912, pp. 348–350.

that the dimorphism of the basidia permits of a closer basidial packing than would be possible if the basidia were monomorphic, and that, thereby, hymenial space is economised for spore-production. The significance of the marked protuberancy of the long basidia in Coprinus fruit-bodies thus became clear for the first time. However, my communication was a brief one and was not accompanied by any illustrations.

In 1914, I gave an account of the structure of the fruit-body of *Bolbitius flavidus* and showed that the mechanism for the production and liberation of spores in the genus Bolbitius is entirely different from that in Coprinus.[1] My observations went to prove that the phenomenon of deliquescence, while of vital importance in securing the free liberation of spores from the pileus of a Coprinus, is only a *post-mortem* change in the fruit-body of a Bolbitius.

In 1914, a fortnight before the Great War broke out, I sent a fully illustrated paper called *The Production and Liberation of Spores in Coprinus sterquilinus* to Germany for publication in Pfeffer's *Festschrift*. This paper, under the direction of the late Professor Klebs, was translated into German and published in the *Festschrift* in 1915.[2] It contains a full account of the details of structure of *Coprinus sterquilinus* together with an expression of my mature views on the organisation of the Coprinus Type of fruit-body. The entirely new points of general interest so far as the genus Coprinus is concerned, which were set out in illustrations for the first time in this paper, are : (1) the dimorphism of the basidia, (2) the fact that in the zone of spore-discharge the long basidia discharge their spores before the short, (3) the wasted spores which cling to the gills particularly at their edges, and (4) the drop of fluid which is excreted at the base of each spore a few seconds before the spore is violently discharged into the air. My observations on the discharge of spores from the basidia of *Puccinia graminis* were also mentioned, and the interesting generalisation was arrived at that

[1] A. H. R. Buller, "The Fruit-body Mechanism of Bolbitius," *Transactions of the British Mycological Society* for 1913, vol. iv, 1914, pp. 235–238.

[2] A. H. R. Buller, "Die Erzeugung und Befreiung der Sporen bei *Coprinus sterquilinus*," Pfeffer's *Festschrift*, identical with *Jahrb. f. wiss. Bot.*, Bd. 56, 1915. For my paper *vide* pp. 299–329, Taf. II and III.

in the Uredineae and Hymenomycetes the mechanism for the discharge of the spores from the sterigmata is exactly the same.

In 1918, I published some critical remarks on the generic positions of *Psathyra urticaecola*, *Coprinus plicatilis*, and *Psathyrella disseminata*.[1] I showed that *Psathyra urticaecola* is really a Coprinus, and therefore renamed it *Coprinus urticaecola* (Berk. et Broome) Buller. An analysis of *Coprinus plicatilis* allowed me to draw the conclusion that this fungus is a true Coprinus and should be retained in that genus. My observations on *Psathyrella disseminata* clearly proved that this fungus is not a Coprinus and that Quélet and Lange were in error in placing it in that genus.

Most of the observations on Coprini which I have recorded in the papers referred to above will be embodied in the next-succeeding Chapters which deal with the various Coprinus Sub-types.

In 1923, Cappelletti,[2] in the belief that the phenomenon of autodigestion had not hitherto been investigated, published a paper called *L'autolisi dell' imenio nel genere Coprinus* in which he treated of the ripening of the spores on each gill from below upwards and the destruction of the gill-tissues from below upwards in several species of Coprinus, his remarks being illustrated by a drawing showing the autolysis of a gill of *Coprinus atramentarius*. In general, his observations confirm my own; but, since he was not aware of my writings on Coprinus, he failed to notice many of the finer details of organisation[3] in the fruit-bodies which he examined. Among his observations are the following. In the hymenium the paraphyses and basidia are strongly united, but they can be separated by subjecting them to Schultze's fluid. From above downwards in a gill the capacity of the cell-walls for absorbing ruthenium-red gradually diminishes, and at the base of a gill there is a boundary line between an upper zone that is colourable and a lower zone undergoing disorganisation which is not. The destruction of

[1] A. H. R. Buller, "Some Critical Remarks on the Generic Positions of *Psathyra urticaecola* Berk. et Broome, *Coprinus plicatilis* Fr., and *Psathyrella disseminata* Pers.," *Trans. Brit. Myc. Soc.*, for 1916, vol. v, 1917, pp. 482–489.

[2] O. Cappelletti, "L'autolisi dell' imenio nel genere Coprinus" (Persoon), *Nuovo Giornale Botanico Italiano*, vol. xxx, pp. 73–93.

[3] *E.g.*, the dimorphism of the basidia, the very narrow zone of spore-discharge, the shooting away of the spores, drop-excretion at the spore-hilum, etc.

the protoplasm in the cells is due to a peptonising enzyme which can be extracted from the gills but not from the stipe. There is more of this enzyme in the upper part of a gill than in the lower. The lamellae contain, in addition, catalases, peroxidases, and traces of oxidases, but no diastase. Lipoid enzymes were not definitely detected. Enzymes capable of destroying the chitin, etc., making up the cell-walls, were not extracted. Bacteria were found among the membranes undergoing destruction, and Cappelletti suggested that the membranolytic action is due to them.[1]

It is true that bacteria may be found contaminating the fluid-products of autodigestion at the gill-edge, but Cappelletti's supposition that normally they are responsible for the breaking-down of the cell-membranes seems to me not only very unlikely on *a priori* grounds but to be disproved by observations made upon pure cultures. In my laboratory very many pure cultures of *Coprinus sterquilinus*, *C. lagopus*, *C. niveus*, *C. narcoticus*, *C. stercorarius*, etc., have been made from spores, in which bacteria have been completely excluded. Yet, notwithstanding the absence of bacteria in these cultures, autodigestion of the gills has proceeded in a perfectly normal manner. Enzymes responsible for destroying the cell-membranes during autodigestion are therefore always produced by the gills themselves.

Critical Remarks on the Generic Position of Certain Agaricineae.—Hitherto there has been a good deal of confusion in regard to the relation of certain Agaricineae to the genus Coprinus. This applies in particular to the genus Bolbitius and to the following species: *Psathyrella disseminata*, *Psathyra urticaecola*, and *Coprinus plicatilis*. The secret of coming to a correct conclusion as to the generic positions of these fungi lies in applying to them as a test the seven criteria which were laid down at the beginning of this Chapter in connection with the definition of the Coprinus Type of fruit-body. Already, in Chapters II and III, by proceeding in this way, I have demonstrated that *Psathyrella disseminata* and *Bolbitius flavidus* each have an organisation for the production and liberation of spores which is entirely different from that of Coprinus; and I have felt justified in asserting, firstly, that the genus Bol-

[1] C. Cappelletti, *loc. cit.*, pp. 88–89.

bitius is sharply marked off from the genus Coprinus, and, secondly, that Quélet and Lange had no sufficient grounds for removing *Psathyrella disseminata* and *P. impatiens* from the genus Psathyrella and transferring them to the genus Coprinus.

Up to the present in this work the generic positions of *Psathyra urticaecola* and of *Coprinus plicatilis* have not been discussed. I therefore propose to analyse the fruit-bodies of these fungi in the

Fig. 54.—*Coprinus urticaecola.* Fruit-bodies coming up on a stick found in Kew Gardens. Spore-discharge about to begin. Photographed at 4 p.m., June 22, 1912. Natural size.

light of the seven already-mentioned criteria. The result will show that the Psathyra must be transferred to the genus Coprinus and that *Coprinus plicatilis* must be retained as a Coprinus. The remarks which now follow are quoted with but slight alterations from a special paper published in 1917.[1]

Psathyra urticaecola.—Whilst hunting for fungi in Queen's Cottage Grounds at Kew, Miss E. M. Wakefield and I found this species growing on sticks (Figs. 54 and 55) and other rubbish which had been dredged from the large lake in the Royal Gardens, and also on dead leaves and haulms of *Holcus lanatus.* On one stick there were from twenty to thirty fruit-bodies which at the time

[1] A. H. R. Buller, *loc. cit.*

were from one-quarter to one-third of an inch in height. At the Royal Herbarium Mr. Massee at once recognised the fungus as *Psathyra urticaecola*, and this view was sustained by other mycologists working there. The correctness of the identification is supported by the original description of the species as given by Berkeley and Broome,[1] which I find to be as follows :

" *A.* (*Psathyra*) *urticaecola*, n. sp. Pileo campanulato, margine striato, stipiteque sursum attenuato, fistuloso, insititio flocculentis albis; lamellis antice ventricosis, postice attenuatis, ex albo spadiceis.

" On nettle-roots. King's Cliffe, Aug. 8, 1858.

" Pileus two lines across, campanulate, flocculent, white : margin at length straight, striate ; stem short, slender, attenuated upwards, flocculent, fistulose, springing immediately from the matrix ; gills ventricose in front, attenuated behind, adnexed, at first white, then a rich chocolate.

" Allied to *A. pennatus*."

The specimens which Berkeley and Broome deposited in the Kew Herbarium are so shrivelled that they were found to be of little or no value in assisting identification of the living fungus : but it was seen that Berkeley had added a note upon the specimen sheet, which contained a description of the species in English similar to that just quoted and also the following additional remark :

" Allied to *A. pennatus* but gills by no means black ; habit of a Coprinus or *A. sericellus*." Finally, there was a pencil note on the sheet showing an oval spore and a description of the spores as " brown with a very slight purple tinge."

The King's Cliffe specimens are illustrated in Plate 596 of Cooke's *Illustrations of British Fungi*, and the size of the spores is there given as $7 \times 4\ \mu$.

From the above description, notes, and illustrations, I was able to convince myself that the fungus which I had collected at Kew was identical with *Psathyra urticaecola* Berk. et Broome.

I discovered that the supposed Psathyra is in reality a Coprinus. The sticks on which the young fruit-bodies were growing were placed

[1] Berkeley and Broome, " Notices of British Fungi," *Ann. and Mag. of Nat. Hist.*, May and June, 1861.

in a damp chamber. The fruit-bodies underwent autodigestion during the following night. Several other crops of fruit-bodies subsequently came up, and each of them shed its spores and became reduced by autodigestion during the night (Fig. 55, *cf.* A and B). On examining the fruit-bodies with the microscope, I found that all the seven criteria already given for the genus Coprinus were satisfied. The gills of the pileus are (1) exceedingly thin, for they scarcely exceed 0·1 mm. in thickness, (2) parallel-sided, and (3) non-geotropic, for they are held together in a solid mass in the same way as in *Coprinus atramentarius*. Since the gills are parallel-sided, non-geotropic, and closely packed, it must of necessity happen that (4) one side of a gill looks slightly upwards and the other slightly downwards. The spores were observed (5) to ripen and (6) to be discharged from below upwards on each gill; and, finally, (7) typical coprinoid autodigestion was seen to take place: the gills were gradually destroyed from below upwards, the spore-free portion being liquefied and thus gradually removed out of the way of the falling spores.

As we have seen, Berkeley remarked on the Kew herbarium sheet that *Psathyra urticaecola* has "the habit of a Coprinus." The reason why Berkeley and Broome failed to discover that this fungus is a Coprinus and not a Psathyra is probably because the autodigestion of the gills takes place at night. I found that the gills are white in the morning but turn chocolate from below upwards with the ripening of the spores during the afternoon (Fig. 54), and that spore-discharge takes place in the evening and night. By the following morning the fruit-bodies are exhausted and falling (Fig. 55, *cf.* A and B).

The spores, like those of several other very small species of Coprinus, are not jet-black but distinctly chocolate-coloured. They measure $7 \times 4\,\mu$ and are oval. The top of the pileus is chalky white and beset with flocculent masses of matted hyphae. The cystidia are cylindric-oval and are firmly fixed by both ends into opposing gills which they bridge as in *Coprinus atramentarius*. The basidia are dimorphic, as is usual for Coprini; but the long and short basidia are rather freely spaced. The paraphyses are distinctly pedicellate.

FIG. 55.—*Coprinus urticaecola*. A, fruit-bodies coming up on a stick found in Kew Gardens. The pilei are beginning to expand and spore-discharge will commence in the course of an hour or two. B, the same stick at 10.45 A.M. the next morning. During the night the gills shed their spores and underwent autodigestion. The stipes of the exhausted fruit-bodies have collapsed. The fine white scales on the surface of the pilei can here and there be readily discerned. Natural size.

The following description of the fungus is based entirely on my own observations :

Coprinus urticaecola (Berk. et Broome) Buller in *Trans. Brit.*

Myc. Soc., 1917, Vol. V, p. 485. Pileus about 5 mm. in diameter in the hemispherical condition, almost spherical at first, becoming hemispherical at the time the spores begin to ripen, opening out and even becoming revolute and radially split during the discharge of the spores, chalky white, unstriated, beset with numerous small white scales composed of matted hyphae unmixed with globular cells and not bearing crystals of calcium oxalate. Gills very thin, about 0·1–0·15 mm. thick, ventricose, maximum width 1·5 mm., at first white, then becoming chocolate, the coloration proceeding from below upwards, held together by cylindrical-ovate cystidia which cross the interlamellar spaces as in *Coprinus atramentarius*, and undergoing complete autodigestion in the evening and night. Stipe when fully elongated 1·5–2 cm. high, 2 mm. thick below, attenuated upwards, scarcely more than 1 mm. thick at the top, white, flocculent at the very base, otherwise smooth, when pulled from the wood to which it is attached leaving a small discoid piece of itself behind, hollow. Spores minute, 7×4 μ, oval, smooth. Spore-discharge and autodigestion of the gills taking place in the evening and night.

On sticks, also on dead leaves and haulms of a grass (*Holcus lanatus*), Queen's Cottage Grounds, Kew, June, 1912.

Coprinus plicatilis.—Massee,[1] in 1906, stated that "many species included in Coprinus, as *C. plicatilis* and others having dry non-deliquescent gills, have no real affinity with this genus"; and, on mycological excursions, I have heard the suggestion put forward that *C. plicatilis* (Fig. 56) is rather a Psathyrella than a Coprinus. The true generic position of this fungus, therefore, appears to be a matter of some doubt.

For a number of years I have had excellent opportunities for examining the structure and mode of spore-discharge of the species in question. I find that all the seven criteria for a Coprinus are satisfied except one: there is no autodigestion of the gills from below upwards. The gills are (1) very thin, for they are not more than 0·15 mm. thick, (2) parallel-sided, and (3) non-geotropic, a fact decided by experiment. Since the gills are parallel-sided and non-geotropic, it must of necessity happen that (4) one side of a gill

[1] G. Massee, *Text-Book of Fungi*, London, 1906, p. 364.

looks slightly upwards and the other slightly downwards. I have convinced myself that (5) the spores ripen in succession from below upwards on each gill and that (6) the spores are discharged in succession from below upwards on each gill; but microscopical as well as macroscopical examination shows that autodigestion does not

Fig. 56.—*Coprinus plicatilis*. Fruit-bodies coming up amid grass under trees. The one on the right is half expanded and the sulcations above its gills are opening out. The one on the left with the depressed disc is fully expanded and is shedding spores. Photographed by Miss E. M. Wakefield and the author in Queen's Cottage Grounds, Kew. Natural size.

proceed from below upwards on each gill. Massee truly described the gills as being dry and non-deliquescent.

I regard *Coprinus plicatilis* as a Coprinus which has lost one of its chief Coprinus characters, namely, autodigestion; but I do not hesitate to retain the fungus in the genus Coprinus, because with this exception all the characters are coprinoid. In no other genus than Coprinus do the spores ripen and become discharged from below upwards on the gills.

The basidia are trimorphic as a rule, but here and there on a gill they may be dimorphic. The long and short basidia, together

with the pavement-like paraphyses, have the normal coprinoid arrangement. The zones of spore-discharge are unusually wide but they nevertheless proceed from below upwards on each gill. The loss of autodigestion is doubtless associated with the fact that the pileus, on opening, becomes spread out like a parasol. The gills split along their median planes from above downwards and thus become Y-shaped in vertical transverse section. The upper parts of the gills thus come to look downwards and the spores there discharged can escape freely from the pileus without any danger of striking an obstacle. It is noticeable, however, that one side of the unsplit lower portion of a gill is often powdered black with spores which have failed to gain their freedom. This appears to be due to the fact that the side so powdered has looked slightly upwards and has therefore caught the spores on its surface after they have been shot away from the hymenium and have fallen vertically for a certain distance. The loss of autodigestion is therefore associated with a certain loss of efficiency in spore-discharge.

In certain other small Coprini, *e.g. Coprinus curtus* (Vol. II, Fig. 32, p. 96), the pileus also opens out parasol-wise before spore-discharge begins and each gill becomes Y-shaped in cross-section. However, in all these species the lower part of the vertical portion of each gill distinctly undergoes autodigestion which can be observed either with the eye or with the microscope, so that here the most characteristic feature of the mechanism of Coprinus fruit-bodies has not been eliminated. My investigations upon numerous small species of the genus Coprinus have convinced me that *Coprinus plicatilis* alone has lost the power of reducing its gills by auto-digestion during spore-discharge; and I therefore cannot agree with Massee when he states that *many* species included in the genus Coprinus have dry and non-deliquescent gills.

There is a fungus which has come up spontaneously on horse dung several times in my laboratory at Winnipeg. It so much resembles the *Coprinus plicatilis* which occurs in such abundance in grassy fields in England, that at first I thought the two species were identical; but a careful comparison has convinced me that they are distinct. The two fungi are similar in their brownish colour when young, in their umbonate disc becoming depressed at

maturity, in the collar formed by the inner ends of the gills around the top of the stipe, and in the black colour, almost identical size, and shape of the spores. However, the Winnipeg species differs from *Coprinus plicatilis* : in (1) having a somewhat browner pileus in the expanded condition, in (2) having a somewhat longer stipe relatively to the breadth of the pileus, in (3) growing on horse dung instead of on grassy turf, but especially in (4) the fact that the lower portion of the vertical part of each gill undergoes a slight but quite definite amount of autodigestion from below upwards during the period of spore-discharge. The Winnipeg species, which seems to be new and as yet undescribed, is evidently a Coprinus and serves to connect *Coprinus plicatilis* with the more typical Coprini, such as *Coprinus lagopus*, *C. niveus*, and others, in which autodigestion of the gills from below upwards is a very obvious and striking character.

Falck's Theory of Radiosensitivity in Coprinus.—Richard Falck,[1] as a result of experiments made with the aid of a lamp, etc., has come to the conclusion that Helvella, Morchella, Gyromitra, and some other Discomycetes in which the hymenium is dark-coloured are *radiosensitive* in respect to the discharge of the spores from their asci, *i.e.* that the fruit-bodies discharge their ripe spores only when their temperature is raised above a certain minimum by heat radiated to them by the sun or some other external heat-source. He thinks that, under natural conditions in the spring, species of Morchella, etc., "shed their spores only in direct sunlight, from sometime in the morning until sometime in the afternoon when the sun has passed its highest point and the period of greatest warming is over."[2] Falck has undoubtedly shown that radiant heat has a remarkable action upon the ripe asci of certain Helvellaceae in that it stimulates them to explode and shoot away their spores ; but, when he attempts to extend his theory of radiosensitivity to the Basidiomycetes and, in particular, to the Coprini, I am unable to follow him.

Under the heading "Arrangements for using the radiation from

[1] Richard Falck, *Mycologische Untersuchungen und Berichte*, Jena, Heft II, 1916, pp. 77–144, Taf. I and II.

[2] *Ibid.*, p. 125.

the sun (Sonnenstrahlung) for the dispersion of the spores of Basidiomycetes" Falck [1] says: "In the Basidiomycetes it is the family of Coprinus-species of which the dispersion of the spores can take place normally only under the influence of radiation. The caps, which in youth are pure white, on ripening take on a dark colour which absorbs radiant energy. I could often observe that the well-known inky deliquescence of Coprinus-species, which results in the flowing away of the majority of the ripe spores,[2] is prevented when the expansion of the pileus takes place under the influence of radiation. In the latter case evaporation of the fluid is so rapid that inky putrefaction does not develop and all the spores become dispersed." In support of these statements Falck, who appears not to have seen any of my publications on the production and liberation of spores in the genus Coprinus, records a few observations on the effect of radiation on the production of spore-deposits by *Coprinus sterquilinus*, *C. atramentarius*, and *C. micaceus*.

Falck's statement that "the dispersion of the spores of Coprinus-species can take place normally only under the influence of radiation" is not well based and can easily be refuted. Moreover, there does not seem to be any good reason for accepting the suggestion that the blackness of the pileus of such a Coprinus as *C. sterquilinus* has an important significance in respect to the absorption and utilisation of radiant energy coming from the sun. The following facts and deductions seem to me to be fatal to Falck's theory of radiosensitivity in so far as the genus Coprinus is concerned:

(1) Large specimens of *Coprinus comatus* [3] and *C. atramentarius* have a continuous spore-discharge period of 24–48 hours, so that they shed vast quantities of spores at night as well as in the day. If they were radiosensitive, they would not shed any spores during the hours of darkness, but observation shows that they do shed spores at this time.

(2) Some species of Coprinus, *e.g. C. lagopus* and *C. macrorhizus*,

[1] Richard Falck, *Mycologische Untersuchungen und Berichte*, Jena, Heft II, 1916, p. 141.

[2] This old conception that during the deliquescence of the gills the majority of the spores become enveloped in the fluid and do not escape into the air is erroneous. *Vide* these *Researches*, vol. i, 1909, pp. 206–208.

[3] These *Researches*, vol. i, 1909, p. 104.

expand their pilei and freely liberate their spores in mushroom-caves where the temperature is very constant and radiation from the sun is excluded.[1]

(3) Certain small species of Coprinus belonging to the *ephemerus* group exhibit a marked periodicity in their development, which is of such a kind that the fruit-bodies shed their spores only during the night. These fruit-bodies, therefore, cannot possibly be radio-sensitive.[2]

(4) *Coprinus sterquilinus* has a continuous spore-discharge period of several hours (8–12 hours in large specimens). A fruit-body begins to shed its spores, not necessarily in the morning, but at any time during the day or night. The spores are shot from their sterigmata into the air between the interlamellar spaces, other things being equal, as well by night as by day. The process of autodigestion of the gills from below upwards takes place both in darkness and in light. The rate of evaporation of the liquid products of autodigestion at the free edges of the gills depends merely upon the hygroscopic state of the atmosphere, the temperature of the air, the speed of the wind, etc. Direct radiation from the sun under field-conditions doubtless warms the pileus, hastens somewhat the speed with which the zone of spore-discharge passes up the gills, and hastens the rate of evaporation from the gill-edges; but such warming is not a necessary condition for successful spore-discharge. In my laboratory at Winnipeg, where *C. sterquilinus* has been grown for some fifteen successive years, the fruit-bodies are never exposed to direct sunlight and yet they discharge their spores into the air in a perfectly normal manner.

The production and liberation of spores in the Coprini is essentially a phenomenon of growth which, like other growth-phenomena, is dependent on temperature. A rise of temperature below the optimum, however caused, hastens the development of the basidia

[1] Observations made by the author in the mushroom-caves at Paris.

[2] Two of these species come up spontaneously on horse dung in my laboratory and I have had them under observation for many years. Also in upwards of one hundred pure horse-dung cultures of *Coprinus lagopus*, made from spores in wide tubes, Mr. W. F. Hanna and I noticed that, under the laboratory conditions prevailing, the fruit-bodies always opened their pilei about midnight and freely shed most or all of the spores before the next day dawned.

and increases the number of spores liberated per minute ;[1] and, when a fruit-body confined under a bell-jar or in a closed chamber is heated by radiant energy coming from a lamp, etc., no doubt convection-currents come into existence and assist in dispersing the spores. However, under natural conditions, there is no reason to suppose that the pilei of Coprini are dependent on direct sunlight for success in producing and liberating their spores, and it seems certain that the wind, which easily swamps small convection-currents formed about a pileus exposed to the sun, is the chief, and by far the most important, agent in spore-dispersion.

[1] *Cf.* these *Researches*, vol. i, 1909, pp. 123–127 ; vol. ii, 1922, pp. 56–57 ; and this volume, chap. xiv.

CHAPTER VII

THE COMATUS SUB-TYPE ILLUSTRATED BY COPRINUS COMATUS

Characters of the Comatus Sub-type—Representative Species—Differences between *Coprinus comatus* and *Coprinus sterquilinus*—*Coprinus comatus*—Additional Illustrations of the Fruit-body—Structure of the Mature Hymenium. Basidial Dimorphism and its Significance. The Functions of the Paraphyses—Tramal Capillarity and the Fate of the Fluid liberated by Autodigestion—*Coprinus comatus* Parasitised by *Stropharia epimyces*—The Production of Fruit-bodies in the Laboratory

Characters of the Comatus Sub-type.—The Comatus Sub-type of fruit-body possesses all the essential characters already described for the Inaequi-hymeniiferous or Coprinus Type : (1) the gills are very thin, (2) the gills are not wedge-shaped but on the whole sub-parallel-sided, (3) the gills are not positively geotropic, (4) usually the hymenium on one side of a gill at maturity looks slightly downwards and that on the other side slightly upwards, (5) the spores ripen in succession from below upwards on each gill, (6) the spores are discharged in succession from below upwards on each gill, and (7) autodigestion proceeds from below upwards on each gill and removes those parts of the gills which have become spore-free and which, if they continued in existence, would become mechanical hindrances to the fall of the remaining spores.

The special characters of the Comatus Sub-type, which enable one to differentiate it from the other Coprinus Sub-types, are as follows :

(1) The gills are not parallel-sided but subparallel-sided and flanged. The two sides of each gill very slightly converge from the pileus-flesh toward the free gill-edge. The edge itself is swollen into a flange which, during the development of the spores, is in

contact with the flanges of adjacent gills. Before the expansion of the pileus, the combined flanges of the gills form a continuous cylindrical sheath just external to the stipe.

(2) Cystidia are absent from the sides of the gills. In this respect we have a marked contrast with the Atramentarius Sub-type.

(3) The interlamellar spaces between successive gills, which are required to render possible the free development of the spores on the hymenium, are secured not by cystidia acting as stays or distance-pieces but, firstly, through the existence of the flanges on the gill-edges, secondly, by an appropriate separation of the gills where they adjoin the pileus-flesh, and thirdly, by the gill-plates being sufficiently shallow and rigid. Here, again, we have a marked contrast with what is found in the Atramentarius Sub-type.

(4) The basidia are dimorphic. They are of two lengths, long and short. In this character there is an agreement with most of the other Coprinus Sub-types, but a difference in respect to the Micaceus Sub-type where the basidia are tetramorphic.

Representative Species.—The Comatus Sub-type was founded on *Coprinus comatus*, but it includes at least one other species, namely *Coprinus sterquilinus*. There can be little doubt that these two species are genetically very closely related to one another. Not only do they agree in the characters just given, but also in a number of others which are of subsidiary importance so far as the mechanism for the production and liberation of spores is concerned. (1) In both species the pileus, just before expansion, is cylindrico-conical and closely covered with white scales. A fruit-body of *C. sterquilinus* at this stage of development is very similar to a small *C. comatus*. (2) Both have a long stipe encircled by an annulus ; and the annulus, usually, is carried upwards during the elongation of the stipe as part of the base of the cylindrico-conical pileus and then dropped upon the stipe at the moment that the pileus begins to open out in a campanulate manner. (3) In both species the stipe contains a thin axial strand of hyphae which runs freely down the centre of its hollow chamber from the disc. (4) In both species, the gills turn pinkish just prior to the ripening of the spores, owing

to the development of a red cell-sap in every cell. (5) In both species, the spores are oval and deep black.[1]

Differences between Coprinus comatus and Coprinus sterquilinus.—These species have been shown to agree in so many details that it is not without interest to point out their differences, which are of particular importance to field mycologists whose chief aim is to differentiate closely-allied species from one another. These differences, as shown by my own observations, are as follows :

(1) As regards habitat. *Coprinus comatus* is not wholly coprophilous. It occurs above turf in grassy fields, and above rubbish including sticks which it is able to rot. The spores germinate on sterilised horse-dung balls in crystallising dishes, and a dense mycelium is produced ; but the mycelium remains sterile even at the end of several months. *Coprinus sterquilinus*, on the other hand, is wholly coprophilous and grows upon horse dung in fields and gardens, etc. The spores germinate on sterilised horse-dung balls in crystallising dishes and produce a copious mycelium which gives rise to fruit-bodies in from four to five weeks.

(2) As regards the colour of the stipe. In *Coprinus comatus* the stipe remains white from first to last. In *Coprinus sterquilinus* the stipe is *at first white, but turns black or blackish at its upper end* during the ripening of the spores and the opening out of the pileus. This blackening is not due to bruising, as Rea [2] erroneously states, or to a spore-deposit. Bruising does not cause a stipe to blacken at the place of injury.

(3) As regards the size of the pileus and stipe. In *Coprinus comatus*, just before expansion, the pileus is usually about 3 inches high and may be as much as 4 inches high ; whereas in *Coprinus sterquilinus*, at the same stage of development, the pileus is usually only about 1·5 inches high, sometimes only 1 inch, and never more

[1] In vol. i, p. 14, I stated that the spores of *Coprinus comatus* at first become pink and then gradually turn black. This was a mistake. The spores are never pink. The gills, it is true, turn pinkish and then black from below upwards. This pink pigmentation, however, is not due to the change of colour in the spores but to the development of a pink colouring matter in the cell-sap of all the cells of the gills, basidia and paraphyses included. The spore-walls, on becoming pigmented, pass through shades of brown to black.

[2] Carleton Rea, *British Basidiomycetae*, Cambridge, 1922, p. 500.

than 2 inches. The stipe of *Coprinus comatus* is both thicker and longer than that of *C. sterquilinus* by about one-third in equally vigorous specimens.

(4) As regards the scales on the pileus. In *Coprinus comatus* the scales on the pileus are coarse, so that they overlap one another and cover most of the pileus-surface. In *C. sterquilinus* the scales are relatively fine, so that they become widely separated during the expansion of the pileus and do not hide the backs of the gills beneath.

(5) As regards the size of the spores. In *Coprinus comatus* the spores are 13·5 μ long and 8·5 μ broad. The spores of *C. sterquilinus* are very distinctly larger, for they are 20–22 μ long and 11–12 μ broad.[1] The basidia of the latter species are also proportionally larger than those of the former.

(6) As regards the number of paraphyses in contact with each basidium. In *Coprinus comatus* the number of paraphyses in contact with each basidium usually varies from six to eight, whereas in *C. sterquilinus* it usually varies from nine to eleven.

Coprinus comatus.—This well-known fungus, which was the starting-point in my investigations of the Coprinus Type of fruit-body, was described in considerable detail in Chapter XIX of Volume I. However, owing to discoveries made since 1909, the description then given needs amplifying. I pointed out that, in this species, the spores ripen and are discharged from below upwards on each gill, and that autodigestion proceeds from below upwards on each gill and destroys those parts of the gills which have become spore-free and which would, if they continued in existence, hinder the fall of the remaining spores. I recognised that the gill-plates, exclusive of the flanges, are subparallel-sided. It is now necessary to add that : (1) the gills are very thin, (2) the gills are not positively geotropic but ageotropic, and (3) usually the hymenium on one side of a gill looks slightly downwards and that on the other side slightly upwards. Thus it is clear that *Coprinus comatus* possesses all the fundamental characters of the Coprinus Type as laid down in the preceding Chapter.

So far as the special characters of the Comatus Sub-type are

[1] These measurements were made in each species with spores of a single spore-deposit.

concerned, I showed in Volume I that in *Coprinus comatus* : (1) the gills are not parallel-sided but subparallel-sided and flanged, (2) that cystidia are absent from the faces of the gills, and (3) that the

FIG. 57.—*Coprinus comatus*. A group of fruit-bodies just before expansion. Photographed at Queen's Cottage Grounds, Kew Gardens, by Miss E. M. Wakefield and the author. Natural size.

interlamellar spaces, which are required to render possible the free development of the spores on the hymenium, are secured by the flange arrangement ;[1] but, unfortunately, like my predecessors, Brefeld, Massee, Patouillard, Boudier, etc., I overlooked the fact

[1] These *Researches*, vol. i, 1909, pp. 196–215.

(4) that the basidia are dimorphic. In my drawings, Plate II, Fig. 12, and Plate III, Figs. 13, 14, 15 and 16, I introduced the long basidia but entirely omitted the short basidia. Short basidia I had seen from time to time ; but, in 1909, I did not think that they were normal constituents of the hymenium and, consequently, left them out. A more careful study of the hymenium of *Coprinus comatus* has taught me that the short basidia even just exceed the long ones in number. The detailed description of the hymenium given in this Chapter will, I trust, finally settle the question of the nature and general arrangement of the hymenial elements.

Additional Illustrations of the Fruit-body.—In Volume I of these *Researches*, a series of illustrations of the fruit-bodies of *Coprinus comatus* was given in connection with a description of the production and liberation of the spores. In order to supplement that series and to facilitate a comparison between *Coprinus comatus* and *C. sterquilinus*, which will be described in the next Chapter, three more illustrations are here provided.

In Fig. 57 is shown a life-size group of four fruit-bodies which were found coming up on the edge of a path in Queen's Cottage Grounds at Kew, England. The stipes are just beginning to elongate and carry upwards the barrel-shaped pilei. Each pileus is covered by the characteristically white overlapping scales, and still has the future annulus attached to its base. The gills within the pileus are beginning to ripen their spores from below upwards ; but, as yet, spore-discharge has not begun, and not even the lowest portions of the gills have been subjected to the process of autodigestion.

In Fig. 58 is shown, in its natural size, the upper two-thirds of a fruit-body with the pileus in an advanced stage of autodigestion. The gills have become considerably shortened from below upwards. Their entirely exhausted parts, which are no longer shedding spores and to which the drops are clinging, are to be seen exposed at the revolute rim of the pileus. The dark drops contain dissolved within them a dark-brown pigment and relatively few spores which have found their way into them by accident. The raising up of the exhausted parts of the gills and flesh by the rolling back of the pileus-rim serves to prevent these structures from being a hindrance to the escape of spores which are continuously streaming away from

beneath the pileus at the rate of about a million a minute. The stout stipe is smooth and white. The fruit-body was obtained

FIG. 58.—*Coprinus comatus*. Upper two-thirds of a fruit-body in an advanced state of autodigestion. Drops of a dark fluid are hanging from the upturned pileus rim. About a million spores a minute are escaping from the gills. Photographed at Winnipeg. Natural size.

growing on soil-covered rubbish at Winnipeg, Canada; and it differed in no wise from fruit-bodies in the same stage of develop-

ment, which the author has frequently observed in various parts of England.

In Fig. 59 is shown, one-third its natural size, a fruit-body which was gathered in a field in England and then set in a vertical position under a bell-jar. As the pileus expanded, spores began to fall. The black spore-deposit upon the paper around the base of the stipe was formed in the course of several hours. About three-quarters of the pileus has disappeared owing to autodigestion, and the useless remnants of the gills and pileus-flesh at the rim are tucked away in the form of a neat roll. An earlier stage of the same fruit-body beneath the bell-jar is shown in these *Researches*, Volume I, Fig. 72, p. 206. These two illustrations afford conclusive evidence that, under natural conditions, the spores of *Coprinus comatus* are discharged into the air and are carried off by the wind and, therefore, that normally they do not become involved, as Massee erroneously supposed, in the liquid products of autodigestion. A full discussion of this matter will be found in Volume I.[1]

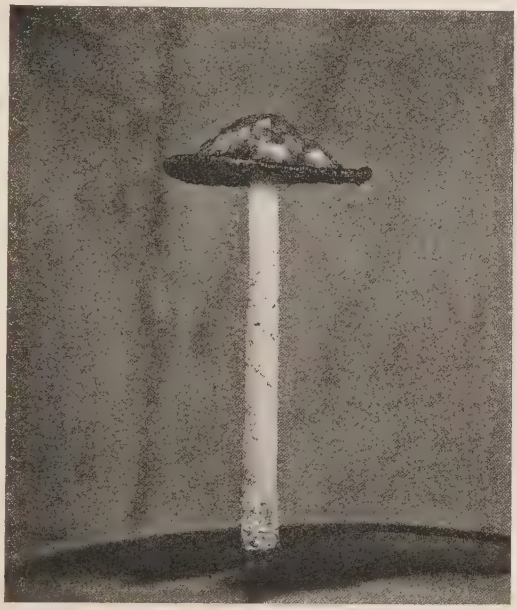


FIG. 59.—*Coprinus comatus*. The liberation of spores. The fruit-body was gathered in a field and then set in a vertical position under a bell-jar. As the pileus expanded, spores began to fall. The black spore-deposit upon the paper around the base of the stipe was formed in the course of several hours. About three-quarters of the pileus has disappeared owing to autodigestion. The rim of the pileus is rolling upwards. An earlier stage is shown in Vol. I, Fig. 72, p. 206. Photographed by P. Grafton. $\frac{1}{3}$ natural size.

Structure of the Mature Hymenium. Basidial Dimorphism and its Significance. The Functions of the Paraphyses.—If, with a

[1] Vol. i, 1909, p. 206.

moderate power of a microscope, one examines the side of a black portion of a long gill of *Coprinus comatus* in face view, one's attention is immediately attracted by the spores; for these bodies are deep black and stand out conspicuously on the white background provided by the general surface of the hymenium. A sketch of the arrangement of the spores over a small area of the hymenium was very carefully made with the help of a *camera lucida* and is reproduced as a plan in Fig. 60, A. The spores, at first, may seem to be dotted about over the area in no particular order, as here illustrated; but, if one focusses up and down with the microscope, one soon discovers that the spores are at two levels, a higher and a lower. Half of the spores are situated on long basidia and the other half on short basidia. The spores which are shown in Fig. 60 at A have been re-sketched at B, so as to bring out the arrangement just described. The spores on the long basidia are all represented in uniform black, whilst those on the short basidia, for the sake of distinction, have been shaded with lines. The black spores must therefore be thought of as standing above the shaded ones. The analysis can be carried further: the two sets of spores can be re-sketched separately. In Fig. 60 at C are shown the spores which are at the higher level in B, and at D are shown the spores which are at the lower level in B.

A glance at the cross-section of a gill represented in Fig. 63 (p. 160) will allow the reader to realise at once the relative heights of the two sets of spores above the paraphyses. From developmental studies which have been made for *Coprinus sterquilinus* and which will be described in the next Chapter we are justified in inferring: (1) that all the basidia which arise in the hymenium of *Coprinus comatus* belong to two generations only, (2) that the long basidia constitute the first or older generation and the short the second or younger generation, (3) that the second generation of basidia begins to develop spores shortly after the first generation has begun to develop spores, (4) that the two generations therefore overlap in their development and are not strictly successive to one another, and (5) that each generation corresponds to a single generation of the Panaeolus Sub-type, such as one may see represented by spores in the dark area in Volume II, Fig. 89, p. 257. Where

convenient, therefore, from now on, we shall speak of the long basidia as the *first generation* of basidia and the short basidia as the *second generation*. It may here be added that the spores on

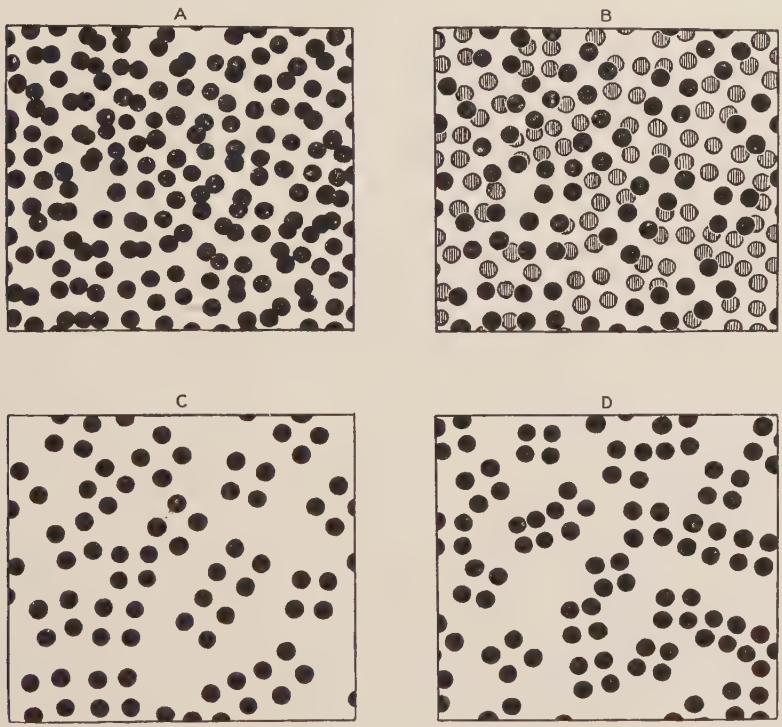


FIG. 60.—*Coprinus comatus*. Dimorphism of the basidia. A, *camera-lucida* sketch of a piece of hymenium 0·15 mm. wide, showing plan of positions of all the spores. B, the same, with the spores of the long basidia shown black and those of the short basidia shaded with lines. C, the spores of the long basidia shown by themselves. D, the spores of the short basidia shown by themselves. Magnification, 293.

adjacent basidia, including both generations, come to have their spores ripe and ready for discharge at very nearly one and the same moment.

It is scarcely necessary to state that in Fig. 60, C and D, each set of four spores corresponds to a single basidium immediately subjacent, and that each spore is borne aloft on a sterigma. From a study of the drawings just mentioned and of Fig. 63, it may be concluded : (1) that the basidia of a single generation are set at

such distances apart that the spores of adjacent basidia cannot touch one another, (2) that the spores of the long basidia are raised so far above the spores of the short basidia that the spores of one generation cannot touch those of the other generation, and (3) that the two sets of basidia are so arranged in relation to one another and to the paraphyses that they form a beautiful mosaic-work. If one considers the area shown in Fig. 60 at C, it is clear that it would be difficult to crowd in more than one or two additional basidia, provided that the new spores were to be at the same level as the others and were to keep a safe distance from neighbouring spores. Yet, owing to the dimorphism of the basidia, all the spores shown at D were actually crowded with perfect safety into this very area, as shown at B. These considerations, based on accurate *camera-lucida* drawings, seem to show in the most conclusive manner that the dimorphism of the basidia is correlated with fruit-body economy. Dimorphism has this advantage over monomorphism: it permits the number of spores which can be developed in safety (*i.e.* without danger of jostling) at one and the same time on any given area to be greatly increased, nay, practically doubled. In a Coprinus, the importance of crowding into any small area of the hymenium the maximum number of ripe spores *at one and the same time* is easily perceived when one recollects: firstly, that the zone of spore-discharge passes from below upwards on each gill once only, so that it traverses a band of the hymenium, say 0·1 mm. wide, in a very few minutes, and, secondly, that autodigestion quickly destroys those parts of the gills which have shed their spores. With the imposition of this time-limit, it is impossible in a Coprinus to increase the number of spores produced on any small area of the hymenium by developing a long series of *strictly successive* generations of basidia, as is done, for instance, in *Panaeolus campanulatus*. The only suitable method for bringing about such an increase seems to be that actually employed, namely, differential protuberancy of the basidia, which in *Coprinus comatus* is expressed as dimorphism, combined with lateral crowding.

When writing the account of *Coprinus comatus* given in the first volume of these *Researches*, I was puzzled to explain the striking protuberancy of the basidia which I had observed; but I had not

then recognised the short practically non-protuberant basidia as regular elements of the hymenium. It is now clear, however, that the protuberancy of the long basidia is directly correlated with the lateral crowding of the basidia taken as a whole, and that it is therefore correlated with an increase in the number of spores produced on the fruit-body.

To sum up the above discussion as it affects *Coprinus comatus*, we may state that the dimorphism of the basidia, associated with lateral crowding, is a beautiful refinement in organisation which, by increasing the number of spores developed on each unit area of the hymenium, makes an important contribution to the efficiency with which the fruit-body carries out its one dominant function of producing and liberating spores.

The area of the hymenium shown in Fig. 60, A and B (p. 153), contains 91 spores belonging to the short basidia and 76 belonging to the long basidia, which indicates that the short basidia on this area numbered 22·75 and the long basidia 19. A similar inequality between the two sets of basidia was found to occur on other areas. The general rule, therefore, is that *on any given area of the hymenium the short basidia slightly outnumber the long basidia*. It may be that this rule is correlated with the relative difficulty of setting the basidia in definitely safe positions so far as the possible jostling of their spores is concerned. Two adjacent short basidia, since their bodies do not protrude beyond the general level of the exterior of the paraphyses, cannot incline appreciably toward one another, whereas two adjacent long basidia, owing to their considerable protuberancy, may not be perfectly perpendicular and may therefore incline toward one another at a greater or less angle. Perhaps, therefore, the more liberal spacing of the long basidia, relatively to the short basidia, is correlated simply with the fact that the longer basidia, relatively to the shorter, require a larger margin of safety to prevent jostling of their spores.

The elements entering into the structure of the hymenium on the sides of the gills are simply the two generations of basidia already described and the paraphyses. Cystidia are not present. The mosaic-work formed by the basidia and paraphyses is well shown in Fig. 61 at A which consists of a plan of a piece of the hymenium

from which the spores have been removed, drawn with the *camera lucida*. The long basidia are shaded, the short left plain, and the positions of the sterigmata are indicated by dots. It will be noticed that the basidia are all isolated from one another, their separation being effected by the intervening paraphyses. From six to eight paraphyses surround each individual basidium. The paraphyses adhere closely together and thus form a continuous system, which may be thought of as a hymenial pavement in which the basidia

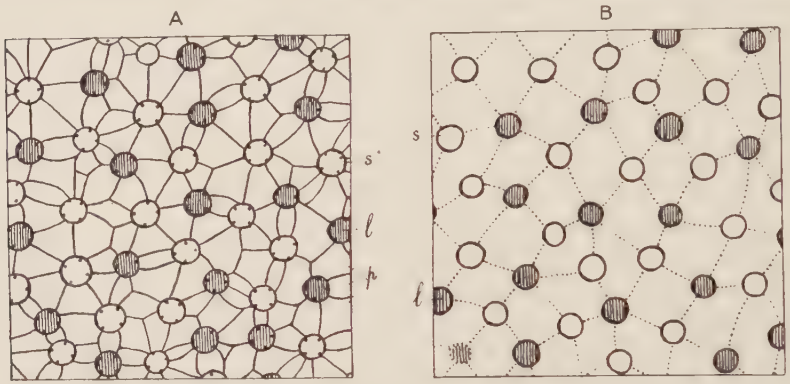


FIG. 61.—*Coprinus comatus*. Arrangement of the dimorphic basidia. A, a *camera-lucida* sketch of a surface view of the hymenium after the removal of the spores. The long basidia, *l*, are shaded with lines, and the short basidia, *s*, are left unshaded. Paraphyses, *p*, separating adjacent basidia. B, a similar sketch to that shown at A but with the paraphyses and sterigmata left out. The dotted *lines of organisation* have been added arbitrarily by the author. In general, along any one of these *lines* there is an alternation of long and short basidia, *l* and *s*. Magnification, 293.

are set at intervals. The paraphyses appear to have several functions to perform: (1) they act as space-making agents for separating adjacent basidia; (2) by gradually increasing in size laterally after the basidial bodies have been formed, they assist in extending the area of each gill and in thus helping to expand the pileus; (3) they give mechanical support to the individual basidia, particularly the long ones which, unless altered in shape, could scarcely stand upright without their assistance; and (4) they probably act as nurses to the basidia, when these are developing, by supplying them with water and food substances. The fact that, in the Coprinus Type of fruit-body, the basidia on any small area of

the hymenium must all bear full-sized spores at one and the same time makes the presence of sterile elements, such as the paraphyses, imperative. If one were to take away the paraphyses and crowd the basidia together so that their bodies were touching, since the four spores on each single basidium require more lateral space in which to spread themselves than the basidium-body, the spores on adjacent basidia would jostle one another and mutually hinder both development and discharge. Nothing but confusion would result, and the beautiful organisation of the hymenium would be destroyed. If one were to remove the paraphyses and leave the basidia in their original positions, the basidial bodies would be naked and unsupported throughout their whole length, in consequence of which they would doubtless have much greater difficulty in keeping their axes perpendicular to the general surface of the hymenium and would be much more likely to suffer, should transpiration of water from their surfaces exceed a certain minimum. I think it probable that the paraphyses are important in helping to secure the proper spatial arrangement of the basidia in the first instance, when the elements of the hymenium are undergoing their initial organisation. The first elements in the hymenium to attain their full lateral extension are the basidia ; and, at the moment when this extension has just been completed, the paraphyses are relatively small and still capable of considerable growth in a plane parallel to the surface of the gill. As the gill gets older, the paraphyses at least double their superficial area. The paraphyses are therefore the elastic elements of the hymenium, and all the later stretching of this structure, which is important in the turning outwards of the gills at the base of the pileus, is accomplished by them. That the paraphyses give mechanical support to the basidia is sufficiently obvious ; and that they pass food materials to them when they are becoming filled with the substances which are destined to be crammed into the spores, and that they supply them with water according to their needs, seems sufficiently probable. The extension of the paraphyses during the development of the hymenium in *Coprinus sterquilinus* will be discussed with the help of illustrations in the next Chapter.

It has been shown that the long and short basidia and the para-

physes are so arranged that they form a mosaic-work pattern. A close inspection of the drawings A and B in Fig. 61 (p. 156), which were made with the *camera lucida*, enables us to formulate the following rules for the distribution of the basidia :

(1) A long basidium is nearer to one or more short basidia than to any other long basidium.

(2) A short basidium is nearer to one or more long basidia than to any other short basidium.

(3) The short basidia are always a little more numerous than the long basidia.

The first two rules might be combined in a single statement running thus : *like basidia tend to repel one another more than unlike basidia.* Another way of putting this is : *like basidia tend to repel one another and unlike basidia to attract one another.*

In Fig. 61, B (p. 156), is shown the exact distribution of the basidia on a particular area of the mature hymenium ; but the paraphyses have been omitted, and certain dotted lines have been added arbitrarily in order to indicate what may be conceived of as *lines of organisation.* One can perceive that, in general, there are two sets of lines which tend to cross one another. If one traces one of these lines across the field, one finds that long and short basidia alternate along it in a fairly regular manner. Occasionally a line bifurcates so that we get two short basidia where, for a regular pattern, only one would have been necessary. In the whole area, as a matter of fact, the long basidia are to the short basidia in the proportion of 100 to 125. It is conceivable, although I have no evidence to offer in support of the idea, that, after the general pattern or arrangement of the elements in the hymenium has been established in the first instance, the paraphyses are subsequently stimulated so that they tend to swell in a greater degree between two like basidia than between two unlike. By this means, adjustments of the pattern of the hymenium might be effected in such a way as to secure the distribution of the basidia according to the rules already formulated ; and thus would be secured the requisite safety for the development and discharge of the spores of every single basidium. The forces at work in bringing such a finely-wrought mosaic-work as the hymenium of a Coprinus into existence, must surely be complex.

Perhaps the foregoing remarks may stimulate someone to elucidate them.

In making *camera-lucida* drawings of the living hymenium, owing to certain optical difficulties, it is not easy to sketch the spores, the basidia, and the paraphyses all at one time. However, a complete surface drawing is represented in Fig. 62. The exact positions of the spores are shown, and they are the same as those already given in Fig. 60, B (p. 153). The basidia and paraphyses have been added semi-diagrammatically. If it were possible, I should remove the corresponding drawing in the first volume of these *Researches* (Plate III, Fig. 15) and should set this one in its place. The old drawing shows only one set of basidia and, as already explained, was made at a time when I thought that the basidia, in general, were all of one length and before the idea of two generations of basidia had come into my mind. The new drawing shows clearly enough the basidia of the first and second generations and the manner in which they are crowded together so that the spores of the former often overlap in part the spores of the latter. Here then, at last, we have a surface view of the hymenium of *Coprinus comatus* which shows, accurately and in detail, the true relations of all the elements. Up to the time of publication of this volume, there was only one other such illustration of a Coprinus in botanical literature, namely, one for *Coprinus sterquilinus* which was drawn by myself to accompany a paper for Pfeffer's *Festschrift*.[1]

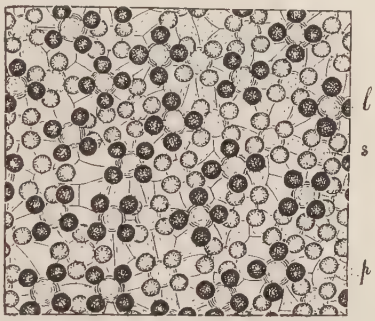


FIG. 62.—*Coprinus comatus*. Surface view of an area of the hymenium 0·15 mm. wide, shortly before discharge of the spores showing all the elements. The positions of the spores were determined with the *camera lucida* and are the same as those shown in Fig. 60. The basidia and paraphyses have been added semi-diagrammatically. The long basidia, *l*, and the short basidia, *s*, along with the paraphyses, *p*, form a beautiful mosaic-work. Magnification, 293.

A semi-diagrammatic drawing representing a cross-section

[1] A. H. R. Buller, "Die Erzeugung und Befreiung der Sporen bei *Coprinus sterquilinus*," Pfeffer's *Festschrift*, identical with *Jahrb. f. wiss. Bot.*, Bd. 56, pp. 299–329, Taf. II and III.

through part of a gill of *Coprinus comatus* is shown in Fig. 63. It is supposed that the spores are all black and therefore almost ripe and ready for discharge. The basidia of the first generation are

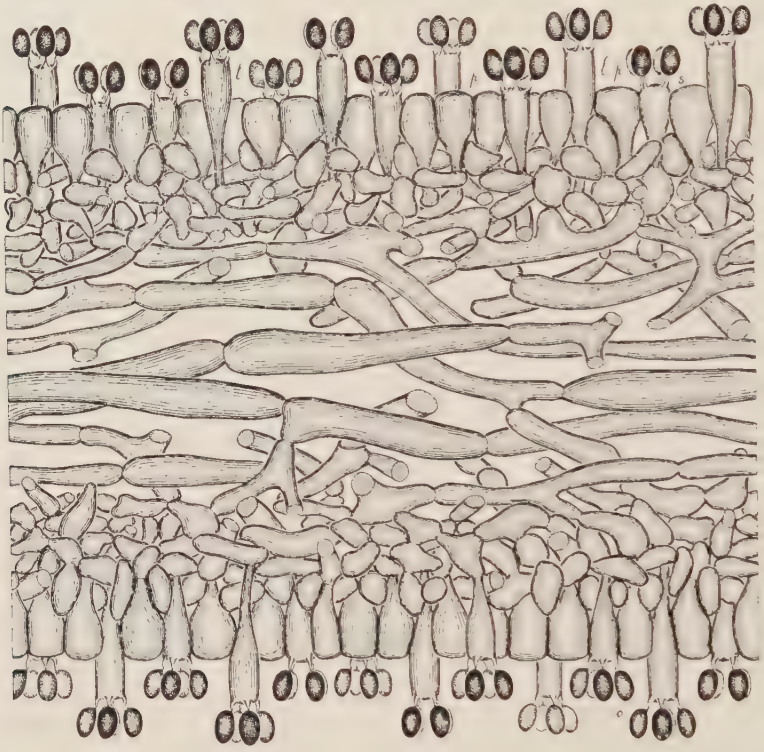


Fig. 63.—*Coprinus comatus*. Dimorphism of the basidia. A cross-section through part of a gill shortly before the beginning of spore-discharge. The long basidia, *l*, and short basidia, *s*, more or less alternate with one another, and are crowded together so that the spores of the former often overtop the spores of the latter, *e.g.* at *o*. The spores of two adjacent basidia are prevented from touching one another owing to the differential protuberancy of the basidia and the presence of the paraphyses, *p*. The loose texture of the trama and subhymenium is also exhibited. Magnification, 293.

markedly protuberant. Their spores are all about the same distance from the general upper surface of the paraphyses and so far above the spores of the second-generation basidia that there is no danger of contact. The basidia of the second generation, which are slightly more numerous than those of the first, are practically non-protuberant: the tops of their bodies are on a level with the outer walls of the paraphyses. Between the basidia are the paraphyses

which, as space-making agents, keep adjacent basidia separated by just such spaces as are required to prevent the spores from jostling. The partial overstanding of the spores of the second generation by those of the first can be observed in several places, notably at *o*. A subhymenium of smaller, shorter, oval cells is more or less differentiated from the trama, which is made up of relatively long and swollen cells. There is no difference in the size of the spores of the two generations or in the appearance of their sterigmata.

Now that it has been demonstrated that there are two generations of basidia in the hymenium of *Coprinus comatus*, the exact manner of spore-discharge in the zone of spore-discharge which moves from below upwards on each gill requires further elucidation. If, in the zone of spore-discharge, the spores of the short basidia were to be shot away before the spores of the adjacent long basidia, it is clear that the former would often strike against the latter, and confusion would result. As a matter of fact, however, this order is reversed : in any small area of the hymenium (0·1 square mm.) the spores of the long basidia are shot away just before those of the short basidia. This refinement of organisation, which can only be mentioned here, will be dealt with more fully in the next Chapter which contains a description of *Coprinus sterquilinus*.

Tramal Capillarity and the Fate of the Fluid liberated by Autodigestion.—With a view to elucidating the fate of the fluid produced by the autodigestion of the gills from below upwards, a series of experiments was made on *Coprinus comatus* fruit-bodies in which the pileus was either (1) about to expand, the gills being white or blackening from below upwards, or (2) already somewhat expanded and campanulate, as shown in Fig. 64, autodigestion being in progress in the lower parts of the gills, and the upper parts of the gills being locked together by their flanged edges.

Drops of water and of red ink were placed on : (1) the scales of the pileus, (2) the outer surface of the pileus-flesh, and (3) the hymenial surface of a number of gills. The drops were not absorbed by the underlying tissues.

Drops of water and of red ink were placed on : (1) the white or pinkish flange of a single gill, (2) the white or pinkish gill-flange sheath composed of a number of gill-flanges united together

(*cf.* Fig. 64), and (3) the wet edge of a gill where spore-discharge and autodigestion were in progress (*cf.* Fig. 64). The drops were all

Fig. 64.—*Coprinus comatus.* Photograph of the under side of a large pileus at a stage when the pileus was campanulate in form and had been shedding spores for some hours. In the centre : the stipe, hollow, and containing a characteristic central white strand. Around the stipe : the gill-flange sheath made up of pinkish gill-flanges in contact with one another. In the outer black zone : the wet autodigesting gill-edges separated by interlamellar spaces through which the discharged spores were escaping in vast numbers. Two drops of fluid produced by autodigestion are hanging from the exhausted rim of the pileus. Similar drops on other parts of the rim were removed with blotting-paper before the photograph was taken. Photographed for the author by Philip Grafton at Birmingham, England. Natural size.

absorbed very rapidly. Small drops of red ink set with a pen on the gill-flange sheath were absorbed into the interior of the gills in 7 seconds. A large drop of water, 5 mm. wide, was absorbed by the sheath in 2·5 minutes and a small drop, 1·5 mm. wide, in 16 seconds. A drop of red ink was absorbed by the autodigesting edge of a gill within a fraction of a minute and colour-changes

showed that it travelled within the gill in the direction of the pileus-flesh for a distance of 2–3 mm. Drops of water were absorbed by an autodigesting gill-edge in the same manner as drops of red ink.

The absorption of drops of water or red ink at the edge of an intact or autodigesting edge of a gill is simply due to capillarity : the water is sucked up into the spaces between the hyphae of the trama (*cf.* Fig. 63, p. 160).

During the autodigestion of a gill, as more and more cells are destroyed, a considerable amount of fluid is constantly being set free. The removal of this fluid from the gill-edge, where its undue accumulation might interfere with the escape of the discharged spores, is brought about in part by evaporation and, as indicated by the experiments just described, in part by capillary suction into the trama. Under dry atmospheric conditions much or most of the fluid is rapidly removed by evaporation, but under very damp atmospheric conditions a relatively small amount of the fluid evaporates, the rest being sucked up into the trama. In the later stages of pilear expansion the gills split radially from above downwards for a certain distance in a Y-like manner, thus exposing to the sky the two halves of the trama. The fluid absorbed by the trama during autodigestion may thus become exposed on the top of the pileus and evaporate there as well as at the free gill-edges. As autodigestion proceeds and the gills become more and more reduced to mere ridges, the fluid which has not been evaporated is set free from the trama and collects in the form of drops at the periphery of the pileus, *i.e.* where the gills have ceased to be active and, therefore, where hanging drops cannot interfere with the escape of the spores.

There is every reason to suppose that the capillarity of the trama is of importance in removing from the gill-edge the fluid produced by autodigestion, not merely in *Coprinus comatus*, but in many other large Coprini, *e.g. C. sterquilinus*, *C. picaceus*, *C. atramentarius*, and *C. lagopus*.

Coprinus comatus Parasitised by Stropharia epimyces.—It is a remarkable fact that in North America the fruit-bodies of *Coprinus comatus* and *C. atramentarius* are sometimes parasitised by another agaric known as *Stropharia epimyces* (Peck) Atk. This parasite was

first described by Peck [1] in 1884 as *Panaeolus epimyces*, but in 1902 and 1907 it was redescribed by Atkinson,[2] and placed by him in the genus *Stropharia* because of the purplish tinge of the spores and the presence of an annulus on the stipe. McDougall,[3] who has studied the development of the fruit-bodies, believes this change of generic position to be justified. The late E. T. Harper [4] held the view that, in all probability, *Stropharia epimyces* (Peck) Atk. is identical with *Pilosace algeriensis* (Fries) Quél.

Stropharia epimyces does not appear to be very rare, for it has been found in the United States: by Peck [5] at North Greenbush (New York State), by Atkinson [6] at Ithaca (New York State), by Goodwin [7] at Port Huron (Michigan), by Sherman and McKenna [8] near Madison (Wisconsin), by O. L. Taylor [9] on Manitoba Island, White Bear Lake (Minnesota), and by McDougall [10] at Urbana (Illinois); and it has also been found in Canada: by Pennington [11] at Winnipeg (Manitoba), by J. H. Faull [12] at Toronto, and by W. S. Odell [12] at Ottawa (Ontario). I myself have not yet found it in

[1] C. H. Peck, "Report of the State Botanist," *N.Y. State Mus. Nat. Hist.* Bull. 133, 1884.

[2] G. F. Atkinson, "Preliminary Notes of Some New Species of Fungi," *Journal of Mycology*, vol. viii, 1902, pp. 110–119; also "A Mushroom Parasitic on Another Mushroom," *The Plant World*, vol. x, 1907, pp. 121–130.

[3] W. B. McDougall, "Development of *Stropharia epimyces*," *Bot. Gazette*, vol. lxvii, 1919, pp. 258–263.

[4] E. T. Harper, "The Probable Identity of *Stropharia epimyces* with *Pilosace algeriensis*," *Mycologia*, vol. v, 1913, pp. 167–169; also "Two Parasitic Mushrooms," *Mycologia*, vol. viii, 1916, pp. 65–71. Harper points out that Lanzi in his *Fungi mangerecci*, etc. (Rome, 1896–1902, Plate 67, Fig. 3), has published an illustration of *P. algeriensis* which, unperceived by Lanzi, appears to show that fungus as a parasite upon the fruit-body of a Coprinus. However, although *C. comatus* and *C. atramentarius* are so common in Europe, no European mycologist has yet recorded finding *P. algeriensis* as a parasite upon them.

[5] C. H. Peck, *loc. cit.*

[6] G. F. Atkinson, 1907, *loc. cit.*

[7] A. W. Goodwin, *vide* C. H. Kauffman's *Agaricineae of Michigan*, 1918, vol. i, p. 254.

[8] Helen Sherman, "The Host Plants of *Panaeolus epimyces*," *Journal of Mycology*, vol. xi, 1905, pp. 167–169.

[9] O. L. Taylor, in Atkinson, 1907, *loc. cit.*, p. 127.

[10] W. B. McDougall, *loc. cit.*; also "Some Interesting Mushrooms of Champaign County," *Trans. Illinois Acad. of Sci.*, vol. ix, 1916, pp. 125–128.

[11] L. H. Pennington, *vide* C. H. Kauffman, *loc. cit.*

[12] Information given verbally.

Manitoba, but I am acquainted with it from living specimens kindly sent me by Professor W. B. McDougall of the University of Illinois.

In Fig. 65, on the extreme left, with a reduction to two-thirds

FIG. 65.—*Coprinus comatus* and its parasite, *Stropharia epimyces* (Pk.) Atk. From left to right: (1) a normal unparasitised fruit-body of *C. comatus* just before expansion; (2) two fruit-bodies of *C. comatus* parasitised by the mycelium of *Stropharia epimyces* and hence stunted in their growth; (3) a similar fruit-body, but with two young fruit-bodies of *Stropharia epimyces* developing at its apex; (4) another *Coprinus comatus* fruit-body with two fully-developed *Stropharia epimyces* fruit-bodies protruding from its apex. The scale shows centimetres. Photographed by W. B. McDougall at the University of Illinois. ⅔ natural size.

the natural size, is shown (1) a normal unparasitised fruit-body of *Coprinus comatus*. Then proceeding from left to right are shown : (2) two fruit-bodies which are parasitised by the mycelium of *Stropharia epimyces* and which in consequence are umbilicate and stunted in appearance, (3) a similar fruit-body with two fruit-bodies of the parasite appearing at its apex as white balls, and (4) another similar fruit-body from the top of which protrude two fruit-bodies of the parasite which are fully developed and are shedding spores.

In Fig. 66, a little less than natural size, is shown a cluster of about twenty fruit-bodies of *Stropharia epimyces* springing from the tops of several large but very much stunted and more or less umbilicate living fruit-bodies of *Coprinus comatus*. The photograph illustrates extremely well the success which the parasite has in mastering its host-plants. The hosts, after being attacked by the mycelium of the parasite, never elongate their stipes or open their pilei. Moreover, although the gills sometimes produce spores, the spores are never liberated, so that the parasitised fruit-body contributes nothing to the reproduction of the species. There can be no doubt that the stunted growth of the Coprinus is largely due to starvation, in that the food materials, collected by the mycelium of the Coprinus and normally destined to find their way into the Coprinus spores, are abstracted from the host fruit-body by the mycelium of the Stropharia and are used to construct the fruit-bodies and spores of the parasite.

The structure of *Coprinus atramentarius* fruit-bodies parasitised by *Stropharia epimyces* has been thus described by E. T. Harper :[1]

" In plants which are parasitised, the elongation of the stem is inhibited and the enlarged gill chambers lie obliquely upward with their mouths outward in the position which they have in the fully expanded carpophore of a mushroom of which the pileus becomes obconic or infundibuliform. Thus is formed the top-shaped mass of the host plant shown in the illustration " (*cf.* Figs. 65 and 66).

" The substance of the veil which is left near the base of the stem in normal plants becomes greatly thickened and enlarged and covers the mouths of the gill chambers up to the margin of the

[1] E. T. Harper, *loc. cit.*, vol. viii, pp. 70–71.

pileus with a thick coat. The base of the stem is also much thickened and does not become hollow. In many cases, there is a solid

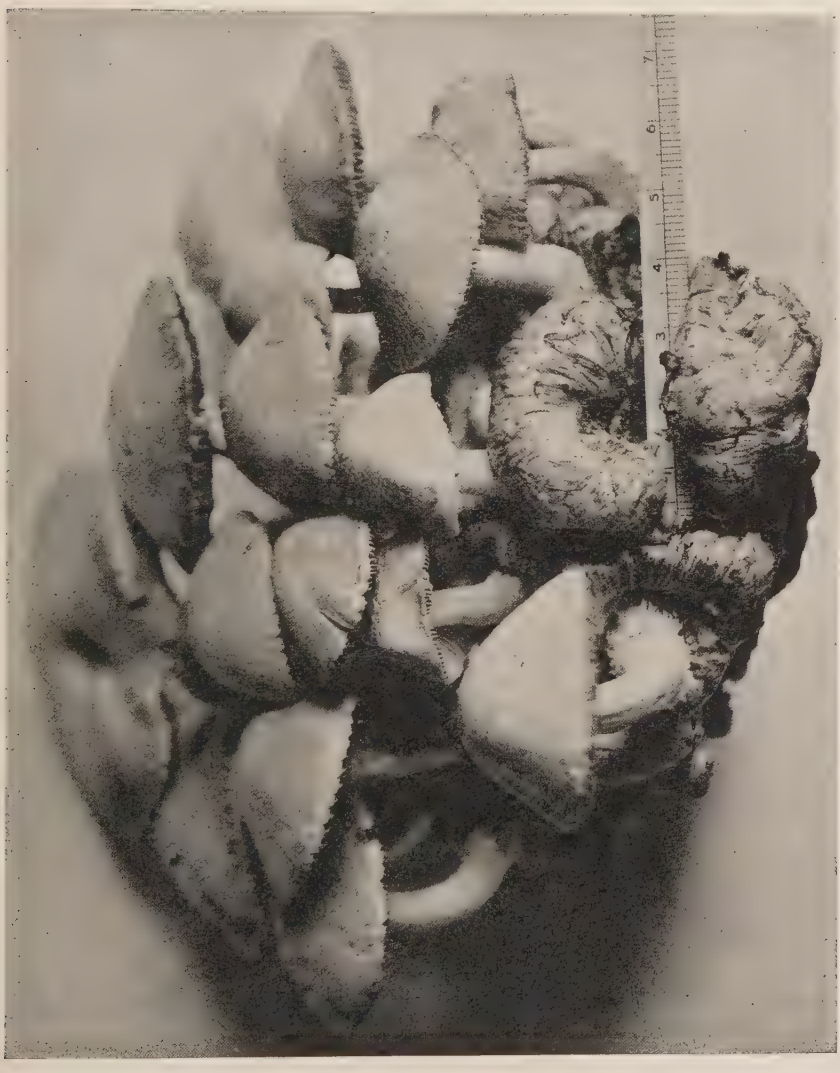


FIG. 66.—*Stropharia epimyces* (Pk.) Atk.—an agaric which is parasitic upon *Coprinus comatus* and *C. atramentarius* in North America. A cluster of fruit-bodies of the Stropharia springing from the living pilei of *Coprinus comatus*. The scale shows centimetres. Photographed by W. B. McDougall at the University of Illinois. $\frac{1}{10}$ natural size.

mass of parasitised stem, veil, and gill tissue and no annular cavity is formed, but often a more or less evident slit appears between the gills and the veil.

" The gills of the host plants attain nearly normal size and some-

times produce spores but are more or less parasitised. The substance of the pileus appears to be least affected and is not greatly thickened in the plants observed. The mycelium of the parasite causes the greatest deformation in the stem and the fruiting bodies are produced from the apex of the stem."

Since the fruit-bodies of *Coprinus sterquilinus*, although smaller, resemble those of *Coprinus comatus* so closely, I thought it might be possible to parasitise them with *Stropharia epimyces*, and therefore made the following experiments. I (1) sowed large numbers of fresh spores of *Coprinus sterquilinus* and *Stropharia epimyces* together on sterilised horse dung in a large crystallising dish, (2) sowed spores of the Stropharia on the mycelial strands and very young fruit-body rudiments of the Coprinus, and (3) inserted spores of the Stropharia into the very young pilei of the Coprinus. However, none of the *Coprinus sterquilinus* fruit-bodies which developed showed the least sign of having been infected by the parasite, for no fruit-body became distorted and all of them shed their spores in a normal manner. Thus my attempts to parasitise *Coprinus sterquilinus* with *Stropharia epimyces* obtained from *Coprinus comatus* completely failed. It would be of considerable interest to find out experimentally, if one could, at what stage of development the fruit-bodies of *Coprinus comatus* and of *C. atramentarius* become invaded by the mycelium of the Stropharia, and also exactly how the mycelium of the parasite is related to its host.

How did the parasitism of *Stropharia epimyces* upon species of Coprini originate ? Our answer, of course, can only be speculative. There appear to be three possibilities :

(1) It is possible that at first the Stropharia, like other Strophariae, lived as a pure saprophyte on dead organic matter of various kinds, that it then became specialised to feed on large dead agarics—just as, at the present time, *Collybia tuberosa* and *C. cirrhata* feed on dead Russulae, Lactarii, Hydna, etc., in the woods at Gimli on Lake Winnipeg and at Kenora on the Lake of the Woods —and that, having taken to a diet of agarics, by some alteration in its excretions it became adapted to grow on living agarics instead of dead ones. This theory would well explain the origin of the parasitism of *Hypomyces lactifluorum* on *Lactarius*

piperatus [1] for the species of Hypomyces are usually saprophytes on dead agarics. However, it does not seem suited to explain the origin of the parasitism of our Stropharia on Coprinus ; for, at the present day, Strophariae saprophytic on dead agarics are unknown.

(2) The second possibility is that originally the Stropharia and a Coprinus came into contact as saprophytic competitors for the same nutrient substratum and that, subsequently, the Stropharia gave up its saprophytic mode of existence and became directly parasitic upon the living Coprinus. A parallel for this second supposition is to be found in the probable origin of the parasitism of Rhinanthus, Bartsia, Euphrasia, etc. These green root-parasites, at first, were doubtless holophytic like the other Scrophulariaceae ; and then, in all probability, they became directly parasitic on the living grass plants and other herbs with which they at first competed on equal terms. It is also probable that Cuscuta changed directly from being a holophyte, like its near relative Convolvulus, to being a parasite.

(3) The third possibility is that originally there was a Stropharia which lived saprophytically on dung or some other dead organic substratum, and that the spores of this fungus underwent an internal physiological change which enabled them to germinate successfully and produce a mycelium, not only on their customary dead organic substratum, but also on a living Coprinus. Thus, owing to a relatively slight, although important, evolutionary saltation, the Stropharia suddenly became a successful parasite. Later on, we may suppose, it became specialised for its parasitic mode of life and ceased to live saprophytically.

It seems almost impossible to explain the manner in which the highly specialised parasitism of the Uredineae came into existence unless one supposes that, from time to time in the course of evolution, the spores of certain species suddenly acquired new powers which enabled them successfully to attack previously immune phanerogamic host-plants. Thus the evolution of parasitism in our *Stropharia epimyces* and in the Rust Fungi may well have been due to the same fundamental factor.

Whatever may have been the exact origin of the parasitism of

[1] *Cf.* vol. ii, 1922, pp. 58–69, Figs. 17–19.

Stropharia epimyces, this fungus conceivably may have become a parasite on the common ancestor of both *Coprinus comatus* and *C. atramentarius* far back in the evolutionary period.[1]

The Production of Fruit-bodies in the Laboratory.—When spores of *Coprinus sterquilinus*, *C. niveus*, *C. lagopus*, *C. stercorarius*, or *C. narcoticus* are sown upon sterilised horse-dung balls set closely in a crystallising dish which is 10 inches in diameter, covered by a glass plate, and kept on a laboratory table at room temperature, the spores soon germinate, the mycelium grows vigorously, and fruit-bodies are developed in from two to five weeks; and similar results can be obtained with *Panaeolus campanulatus*, *Anellaria separata*, *Stropharia semiglobata*, and several other coprophilous Hymenomycetes. *Coprinus sterquilinus* invariably fruits about a month after the sowing of the spores. Now, since the fruit-bodies of *Coprinus comatus* are very similar in structure to those of *C. sterquilinus*, it seemed not unlikely that *C. comatus* would fruit in crystallising-dish cultures. I therefore sowed the spores of *C. comatus* on dung in the usual way. The spores germinated, the mycelium grew vigorously and spread throughout the substratum, and clamp-connections appeared upon the hyphae; but, although the mycelium was kept moist and remained living for upwards of a year, it never produced any fruit-bodies whatever. This experiment was repeated a second time with similar results. It was evident that I had failed to provide the mycelium with the right conditions for sporophore production.

Dr. Roland Thaxter has informed me that he once sowed spores of *Coprinus comatus* on sterilised dung with a result similar to my own: the mycelium grew well but never fruited; and, as we shall see, Miss Mounce could never obtain the fruit-bodies of this fungus from ordinary dung cultures. However, Brefeld,[2] in a couple of lines in his *Untersuchungen*, makes the statement that the large fruit-bodies of *Coprinus comatus* and *C. atramentarius* are very easily raised in dung cultures; but he gives no particulars of his culture methods or of the time elapsing between the sowing of the spores

[1] For some other remarks on *Stropharia epimyces*, *vide infra*, chap. xiii.

[2] O. Brefeld, *Untersuchungen über Pilze*, Heft VIII, 1889, Autobasidiomyceten, p. 39.

and the development of new sporophores. The negative results with ordinary dung cultures obtained by myself, Dr. Thaxter, and Miss Mounce do not support Brefeld's statement that the fruit-bodies of *Coprinus comatus* can be easily raised in dung cultures. It is therefore all the more to be regretted that this distinguished mycologist should have dismissed his successful experiments with the two largest Coprini in so few words.

At Winnipeg, large numbers of *Coprinus comatus* fruit-bodies appeared upon a small lawn and a cinder path in front of a house. The owner of the house, who held the erroneous belief that the fruit-bodies were poisonous, desired to prevent their coming up ; so he raked over the cinder path and dug up part of the lawn. Nevertheless, after a while, both on the path and on the lawn, large numbers of new fruit-bodies sprang into existence. The owner of the house, thus finding his authority mysteriously defied, asked me to come and see the fungi and advise him how he might free his premises from what he evidently regarded as a plague. On visiting the house, I found the fruit-bodies in dense clusters scattered over the lawn, some of them with their shaggy barrel-shaped pilei 4 or 5 inches high and in that interesting stage when they are just beginning to expand and shed their spores. As we looked at the fruit-bodies, the owner said to me : " What would you do with them ? " " Eat them," was my prompt reply ; but he looked at me half incredulously and appeared to be wondering how any one could swallow so dangerous a thing as a Toadstool and ' get away with it.'

To find out upon what the *Coprinus comatus* had been feeding, with the help of a spade I dug a hole in the lawn to a depth of 3 feet. It soon became evident that the mycelium had been growing upon laths of wood, horse dung, and other rubbish which had been buried beneath the lawn a few years before at the time the house was built. I traced the mycelium from the fruit-bodies downwards through the ground for a distance of 34 inches. Thus it was proved that the mycelium of *Coprinus comatus* can grow on substrata buried at a considerable depth beneath the soil, and then produce fruit-bodies at the soil's surface.

On another occasion, I found a cluster of *Coprinus comatus* fruit-bodies above bare cinders on a railway track. Upon removing

the cinders with a stick, I satisfied myself, from the absence of wood or dung, etc., in the cinders removed, that the mycelium must have vegetated in sticks, leaves, or some other nutrient substratum, buried beneath the cinders at a depth of more than one foot, and, subsequently, must have grown upwards through the cinders to the surface of the ground where the fruit-bodies had made their appearance.

Miss Irene Mounce, when investigating the production of Coprinus fruit-bodies from monosporous mycelia in my laboratory, made ten pure cultures of *Coprinus comatus* on horse dung in large crystallising dishes similar to those already described. Two of the dishes were inoculated with many spores (polysporous cultures) and the other eight with hyphae taken from the inside of a stipe of a freshly gathered fruit-body. In every dish a heavy white mycelium was produced which, in the course of a month, completely covered the dung; yet the cultures, like those previously made by myself, remained entirely sterile. Several of them produced thick mycelial cords at the bottom of the culture dish, and one developed woolly knots on the cords; but even rudimentary fruit-bodies failed to make their appearance.

Various changes in the conditions of cultivation of the fungus were made by Miss Mounce in an attempt to bring about fruit-body production. Some of the mycelia were placed in the dark, one in bright sunlight, and the rest in diffuse daylight: but none of them fruited.

Richard Falck[1] has observed that fruit-body production in *Merulius domesticus* is accelerated by a series of transfers: as soon as the mycelium has covered the medium, a piece of mycelium is transferred to a second vessel filled with the same medium, and from the second vessel a similar transfer is made to a third vessel, and so forth. Miss Mounce made a series of such transfers with *Coprinus comatus* mycelium. A stipe culture was growing on sterilised horse-dung balls in a crystallising dish 5 inches in diameter. As soon as the mycelium had covered the culture medium, a small piece of

[1] R. Falck, "Die Fruchtkörperbildung der im Hause vorkommenden holzzerstörenden Pilze in Reinkulturen und ihre Bedingungen," *Mycol. Unters. u. Berichte*, Jena, Heft I, 1913, p. 62.

mycelium-covered dung was removed and used to inoculate the sterilised dung in a second crystallising dish. Four such successive transfers were made. The mycelium in each culture grew well and covered the medium in 10–11 days. Moreover, its hyphae bore large numbers of clamp-connections, thus showing that it was in the secondary condition. Yet, although the cultures were kept moist for several months, they never fruited.

After these failures, I suggested to Miss Mounce that fruit-bodies might develop in the laboratory if the culture medium, instead of being exposed directly to the air, were buried beneath a thick layer of soil ; for it was under such conditions, as already recorded, that I had observed fruit-bodies appearing in the open. Accordingly, Miss Mounce made two new soil-covered cultures in the following manner.

(1) A glass specimen jar, 11 inches high and 3 inches in diameter (Fig. 67), was filled to a depth of 3 inches with a layer of horse dung mixed with sawdust. This culture medium was packed down solidly, and then the jar was filled to within an inch of the top with sifted black soil. The whole was then covered with a glass plate and sterilised in flowing steam for an hour on each of three successive days. The inoculation was effected with mycelium from a stipe culture. Two holes were made through the soil with a sterilised glass rod, and through them small pieces of mycelium-covered dung were pushed well down into the mixture of dung and sawdust in the base of the jar. (2) A broad 3-litre beaker was filled to within 3 inches of the top with a mixture of dung and sawdust similar to that used for the first culture ; and then the medium was covered with a 2-inch layer of sifted black soil. The whole, after being covered with a glass plate, was sterilised and inoculated in the manner already described for the specimen-jar culture. The two cultures, after inoculation, were set under large bell-jars on a table near a window in the laboratory.[1]

After inoculation the mycelium in the two glass vessels grew

[1] These and other particulars concerning Miss Mounce's cultures are here given partly from Miss Mounce's notes embodied in a paper called " The Production of Fruit-bodies of *Coprinus comatus* in Laboratory Cultures," published in the *Trans. Brit. Myc. Soc.*, 1923, and partly from my own direct observations.

vigorously. In the specimen jar, it completely encircled the culture medium in the course of twenty days, it sent out fine hyphae through the soil, and grew well in the holes in the soil made for inoculation purposes. In the large beaker, it completely encircled the culture medium in six weeks and also invaded the soil as in the other culture. Somewhat later, in both cultures, mycelial strands appeared above the soil and just below it by the glass, and some woolly knots appeared in the strands; but at this time there was no development of fruit-bodies.

The inoculations were made in November, 1921. The first sign of fruiting appeared nine months later; for, toward the end of September, 1922, at a time when wild fruit-bodies of *Coprinus comatus* were coming up on the paths of the botanical garden a few yards away, a tiny sporophore rudiment was observed by Miss Mounce on the surface

FIG. 67.—Production of a normal-sized fruit-body of *Coprinus comatus* in the laboratory. Below, in the jar, the mycelium in a sterilised mixture of horse dung and saw-dust. Above, a thick layer of black soil. The glass plate, which covered the top, now removed. Inoculation with mycelium January 7, 1921. Fruit-body photographed late in September, 1922, by Irene Mounce. Reduced to ½ natural size.

of the soil in the specimen jar. It developed rapidly into a very large normal fruit-body, possessing all the usual *Coprinus comatus* characters (Fig. 67). It elongated its stipe, expanded its pileus, underwent autodigestion and, within a week from the time it was first observed, shed vast numbers of spores. Spores taken from the black spore-deposit were found to germinate readily in potato-agar and somewhat less readily in dung-agar.

About three weeks after the fruit-body just described had shed its spores, many white mycelial knots appeared on the outer surface of the soil, a few centimetres below the upper surface; and ten days later three new fruit-bodies, which had developed in tiny crevices beneath the surface of the soil, pushed their way up through the soil into the air. They were creamy-white in colour, somewhat conical in form, and had a smooth, gelatinous, greasy-looking surface. At their base, they were 0·5 cm. in diameter. Five days later they were 7 cm. high, and each fruit-body now showed a distinct division into stipe and pileus. The pilei, which were in general cream-coloured but a deep buff at their

FIG. 68.—A laboratory culture of *Coprinus comatus* with a second crop of three fruit-bodies about to expand their pilei. Below, in the jar, the mycelium growing in a sterilised mixture of horse dung and saw-dust. Above, thick layer of black soil. The glass plate, which covered the top, now removed. Inoculation with mycelium January 7, 1921. Fruit-bodies photographed in October, 1922, by Irene Mounce. Reduced to about $\frac{1}{3}$ natural size.

apices, were now becoming scaly. After five further days the fruit-bodies had reached the stage of development shown in Fig. 68. From an examination of the photograph it is evident that these fruit-bodies, developed in an artificial culture in the laboratory, had the scaly pileus, free annulus, stature, and general appearance of typical wild fruit-bodies. The gills of the fruit-bodies, at the time the photograph was taken, were turning pink from below upwards, and soon thereafter they began to turn black from below upwards. Then each pileus expanded and shed spores in the usual manner.

The beaker culture behaved like the specimen-jar culture. It first developed a single fruit-body (five days before the three fruit-bodies shown in Fig. 68) and then about a fortnight later three more fruit-bodies.

To permit of the upgrowth of the young fruit-bodies, the glass plates covering the cultures were removed. Unfortunately, this led to the infection of the mycelium by maggots and ultimately to its destruction.

Summing up, a total of four fruit-bodies was obtained in each of Miss Mounce's two cultures. It has therefore been demonstrated that, under suitable conditions, *Coprinus comatus* can be brought to the fruiting stage in the laboratory.

CHAPTER VIII

COPRINUS STERQUILINUS

Introduction—Synonyms—Occurrence—Cultures—Description of the Maturing Fruit-body—The Structure and Development of the Hymenium—The Spores—The Mode of Fall and the Adhesiveness of the Spores considered in Relation with Herbivorous Animals—The Discharge of the Spores and the Phenomenon of Autodigestion—Conclusion and Discussion of the Probable Steps in the Evolution of the Inaequi-hymeniiferae

Introduction. — *Coprinus sterquilinus*, as pointed out at the beginning of the last Chapter, is a representative of the Comatus Sub-type and is closely related to *Coprinus comatus*. Its fruit-bodies are large and handsome. Moreover, in contrast with those of *Coprinus comatus*, they can be raised with great ease in pure cultures. I have grown the fungus in my laboratory for several successive years, and have thus been enabled to observe and investigate the structure and development of large numbers of fruit-bodies, with the result that I have succeeded in making out the mechanism for the production and liberation of spores in detail. I now propose to describe this mechanism as fully as possible. Owing to the similarity in organisation of *Coprinus comatus* and *C. sterquilinus*, it will be necessary to repeat with but little alteration for the latter species a number of statements which have been made for the former. However, the account of *Coprinus comatus* was broken, unfortunately, into a main portion in Chapter XIX of Volume I and a supplemental portion in the preceding Chapter of this volume. The account of *Coprinus sterquilinus*, on the other hand, will have the advantage of continuity and will be complete from the first.

In 1915, in a paper in the *Festschrift* published in honour of Wilhelm Pfeffer, in whose laboratory I once had the privilege of

studying, I described the production and liberation of spores in *Coprinus sterquilinus*.[1] What is now about to follow is an elaboration of that paper containing many additional illustrations. It will constitute the first full account of *Coprinus sterquilinus* published in the English language.

To cytologists, histologists, and physiologists, who desire to carry out detailed investigations upon a species of Coprinus, *Coprinus sterquilinus* is to be especially recommended, owing to the ease with which it can be cultivated, the considerable dimensions of its pileus, and the unusually large size of its basidia and spores. In one of the fruit-bodies grown in my laboratory the spores measured 22 μ long and 11–12 μ wide. The size of the basidia and other gill elements is shown by the scale in Figs. 104 and 105 (pp. 242 and 249).

Synonyms.—*Coprinus sterquilinus*, although so well-marked a species, has been described several times under diverse names: by Bolton[2] as *C. oblectus*, by Lindblad[3] as *C. stenocoleus*, and by Peck[4] as *C. macrosporus*. Some systematists, *e.g.* Carleton Rea,[5] still include *C. oblectus* in their works as an independent species, but there is no sufficient justification for so doing. The pilei of *C. sterquilinus* are at first white and subsequently black; but, in changing from white to black, they temporarily become pink.

[1] A. H. R. Buller, "Die Erzeugung und Befreiung der Sporen bei *Coprinus sterquilinus*," *Jahrb. f. wiss. Bot.*, Bd. 56 (Pfeffer's *Festschrift*), 1915, pp. 299–329, Taf. II. and III. This paper was sent to Germany just before the outbreak of the Great War and was translated into German under the direction of the late Professor Klebs.

[2] J. Bolton, *History of Funguses growing about Halifax*, Appendix, 1791, p. 142, Plate 142. Bolton's illustration is reproduced by M. C. Cooke in Plate 661 of his *Illustrations of British Fungi*. Bolton's younger fruit-bodies are correctly coloured, but his older ones, at the stages shown, should have been blackish and not red.

[3] In Fries' *Monographia Hymenomycetum Sueciae*, Upsaliae, vol. ii. 1863, p. 306. For an illustration of *C. stenocoleus vide* E. Fries, *Icones selectae Hymenomycetum nondum delineatorum*, Holmiae et Upsaliae, vol. ii, plate 140, Fig. 1. That the fungus there illustrated is *C. sterquilinus* I have no doubt whatever. Ricken (*Die Blätterpilze*, Leipzig, Bd. I, 1915, p. 57) and Kauffman (*The Agaricaceae of Michigan*, vol. i, 1918, p. 11) hold the same view.

[4] Peck (*31 Report New York State Museum*, p. 35) described *C. macrosporus* from the State of New York. With Kauffman (*loc. cit.*) I agree that the description exactly fits *C. sterquilinus*.

[5] Carleton Rea, *British Basidiomycetae*, Cambridge, 1922, p. 501.

I have no doubt that Bolton based his description of *C. oblectus* on some fruit-bodies of *C. sterquilinus* observed in the pink stage.[1]

Cooke in Plate 660 of his *Illustrations of British Fungi* shows the general size and form of some unexpanded fruit-bodies of *C. sterquilinus*, but the scales are far too dark in colour; they should have been white or whitish. An excellent coloured illustration of an expanded fruit-body, under the synonym *C. stenocoleus*, is provided by Fries in Plate 140 of his *Icones*.

Occurrence.—*Coprinus sterquilinus* is coprophilous: it grows on horse dung. In England I have met with the fruit-bodies on horse dung scattered over a field near Birmingham, on horse dung covered with soil on a path in a wood near Stourbridge, on horse dung placed on one of the flower beds in Kew Gardens, and on horse-dung scrapings partly dug into the soil of a garden near London. Horse dung infected with the mycelium has also been sent to me from Wales. In Canada, I have seen the fungus coming up on horse-dung balls partially buried in a garden on the campus of the University of Manitoba. In the neighbourhood of Winnipeg, *Coprinus sterquilinus* must be very common, for it has come up spontaneously in my laboratory upon horse-dung balls collected from the city streets, every year for the last twelve years and more than twenty times altogether. Massee states that the fungus is found in Britain, France, Germany, Spain, Portugal, Sweden, and Belgium. Possibly its distribution coincides with that of the horse.[2]

Cultures.—I have obtained *Coprinus sterquilinus* on several occasions by taking advantage of the fact that the spores can pass through the alimentary canal of horses without injury. The winter conditions of Manitoba are severe: the ground is usually covered with snow from the middle of November to the end of March, and, during this time, the temperature ranges from 0° to — 40° C. Horse-dung balls dropped in the streets upon the snow in the depth of winter usually become frozen solid in a few minutes, and in this condition they remain until the spring thaw sets in.

[1] Possibly Massee's *C. gigasporus* (*Annals of Botany*, vol. x, 1896, p. 157, fig. 3), although not described as having an annulus, is also synonymous with *C. sterquilinus*.

[2] G. Massee, "A Revision of the genus Coprinus," *Annals of Botany*, vol. x, 1896, p. 139.

The faeces of horses always contain, in addition to innumerable bacteria, the spores of a number of coprophilous fungi. These spores have been swallowed with the fodder and, notwithstanding their passage through the alimentary canal of the animal concerned, have retained their vitality. When, therefore, the dung-balls freeze in the streets of Winnipeg, the spores of various coprophilous fungi which are included in them freeze up too. It was found that, if frozen dung-balls such as those just described are collected from the streets at any time during the winter, brought into the

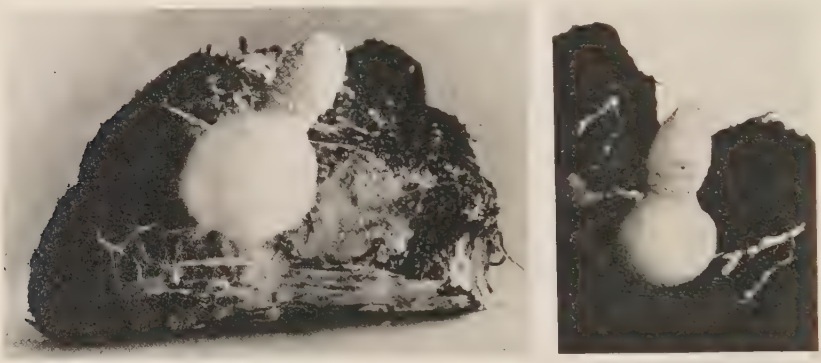


FIG. 69.—*Coprinus sterquilinus*. Two fruit-bodies in a very early stage of development, growing on sterilised horse dung. White mycelium is to be seen on the surface of the dung. Each fruit-body consists of a stipe which is swollen below, and of a conical pileus. Natural size.

laboratory, and placed in a covered crystallising dish, in the course of a few weeks they always give rise to fruit-bodies of one or more small species of Coprinus, of which *Coprinus curtus*, *C. ephemerus*, and *C. lagopus* are the commonest. Sometimes, however, they give rise to the relatively large fruit-bodies of *Coprinus sterquilinus*. It is always possible to know whether or not a dung-ball which has been kept a few weeks is going to produce a fruit-body of this fungus; for, if it is, a white floccose layer of mycelium first appears on the outside of the dung-ball in such a way as to cover the ball in part or as a whole (*cf.* Figs. 69, and 71, p. 182). Further, if several adjacent dung-balls are infected, thick white strands are developed which connect the mycelium of the different balls together. One dung-ball seldom gives rise to more than one fruit-body, and

very often a single fruit-body drains several dung-balls for its substance. The presence of the superficial layer of persistent mycelium and of the mycelial cords is evidently correlated with the fact that the fungus produces large fruit-bodies from a substratum which is apt to be more or less divided into small portions. To gather into a single vegetative body sufficient nutrient matter to produce a sporophore of average size for the species, an apparatus has been developed which unites the mycelium present in several dung-balls into a single system.

Fig. 70.—*Coprinus sterquilinus*. A young fruit-body leaving its annulus as a volva attached to the stipe just above the bulb. Natural size.

To make a culture of *Coprinus sterquilinus* free from other fungi is a simple matter, when once a fruit-body has been obtained. My procedure was usually as follows. About twelve or fifteen fresh horse-dung balls were placed in a crystallising dish (7 inches wide and 2·5 high), so that they covered the bottom. The dish, covered with a glass plate, was then placed in a steam steriliser and kept at a temperature of 100° C. for forty-five minutes. It was then removed from the steriliser and allowed to cool. Inoculation was brought about by raising the cover of the dish and holding a pileus, which was discharging spores, just above each dung-ball for a few seconds. Various precautions were taken to prevent the spores of other fungi from settling on the dung during this operation. The spores invariably germinated and, after a few days, the mycelium grew vigorously. From such a primary culture as that just described new cultures were often made by transferring pieces of the dung covered with the mycelium. Occasionally a

primary culture was made by rubbing sterilised dung-balls with the spore-bearing gills. Still another method of inoculation was employed in which the stipe was used as the agent. The basal part of the stipe remains living for some hours after the pileus has perished. It was found that, if the basal part of a stipe of a nearly exhausted fruit-body is divided up longitudinally into several pieces with a clean scalpel and each piece placed between two sterilised dung-balls, inoculation readily results from the hyphae. Even better results were obtained with the young stipes of unexpanded fruit-bodies.[1]

Fig. 71.—*Coprinus sterquilinus.* A fruit-body with an elongating stipe. The annulus is being carried upwards attached to the base of the pileus. Natural size.

The whole of the life-history from discharged spore to discharged spore was usually run through in the horse-dung cultures in from four to seven weeks. Naturally there were considerable variations in the length of this period, owing to differences in size, shape, proximity, texture, contents, and water-supply of the dung-balls and owing to variations in the temperature of the laboratory. The most rapid development from spore to spore was accomplished in twenty-three days. In this instance the culture was sterilised by placing it in the steam-steriliser three times on three successive days for one hour each time at 100° C.; inoculation was effected by allowing spores to fall on to the surface of the dung-balls from a pileus held above

[1] The stipe-method of making pure cultures was worked out by B. M. Duggar. *Vide* "The Principles of Mushroom growing and Mushroom spawn-making," *U.S. Dept. of Agric., Bureau of Plant Industry*, Bull. No. 85, 1905.

them for fifteen seconds ; and the temperature of the laboratory was maintained at about 20° C.[1]

The most rapid and least troublesome way of obtaining a fresh crop of fruit-bodies is by inoculating the sterilised dung-balls with pieces of dung bearing a vigorously growing mycelium. Cultures made in this way and kept at a temperature of about 20° C. usually produced mature fruit-bodies within thirty days and, under the most favourable conditions, at the end of twenty days. By using the mycelial method of inoculation in place of the spore method, the saving of time in the production of new fruit-bodies was at least a week.

Description of the Maturing Fruit-body.—The young fruit-body begins its development as a rudiment upon the mycelium clothing the exterior of a dung-ball. At first it is merely a globular weft of hyphae ; but, as it increases in size, it becomes compact within and assumes a conical form. The upper part of the cone becomes yellowish and marked off from the lower part by a slight constriction. The lower part is the solid stipe-base, the function of which will be discussed in Volume IV : in most of my crystallising-dish cultures it soon assumed a more or less globular form (Fig. 69, p. 180). The upper part of the cone is the rudimentary pileus enclosing the rudiment of the stipe-shaft. The pileus, after being brought into the light by the growth of the stipe-base, is barrel-shaped (Figs. 69 and 70). About a week after the pileus has begun its development in the light, the stipe-shaft elongates, the pileus expands, and the spores are liberated into the air.

The upper part of the pileus in its youngest state has a smooth greasy-yellow appearance but, as the pileus increases in size, this appearance becomes confined to the disc where it persists until the fruit-body collapses. The sides of the young pileus become broken upon the exterior into small scales and, before the stipe begins to elongate rapidly and shortly afterwards, they are snowy

[1] Since this was written Miss Irene Mounce, working in my laboratory with large test-tube cultures, has observed that the life-history of *Coprinus sterquilinus*, from spore to spore, is usually completed in 24–31 days. *Vide* I. Mounce, " Homothallism and the Production of Fruit-bodies by Monosporous Mycelia in the Genus Coprinus," *Trans. Brit. Myc. Soc.*, vol. vii, 1921, p. 203.

white (Figs. 69, 70, and 71, pp. 180, 181, and 182). As soon as the stipe has attained a length of about 8 cm., the white changes

Fig. 72.—*Coprinus sterquilinus.* A fruit-body with an elongating stipe upon which an annulus has just been deposited from the base of the pileus. The pileus and the upper part of the stipe have turned pink. Natural size.

Fig. 73.—*Coprinus sterquilinus.* A vertical section through a fruit-body with a pileus about to expand. The spores have ripened so that the gills appear black. There is a free filament of loose hyphae running down the middle of the stipe. Natural size.

to silvery-grey. A little later the silvery-grey colour becomes converted gradually into pink (Fig. 72). The blushing of the pileus extends itself to the upper exposed parts of the stipe and is due to the development of a red pigment in the cell-sap throughout the affected parts. The red pigment was found to be present in

the cell-sap of all the cells of the gills, including the basidia and the paraphyses. Its formation precedes the development of the black pigment of the spore-walls. A similar formation of a red pigment in the cell-sap of all the cells of the gills occurs in *Coprinus comatus*. In that species, as one may readily observe, the gills turn pink from below upwards: the upward-moving pink zone is followed by an upward-moving blackening zone, the latter owing its origin to the pigmentation of the spores. Under moist conditions, when the stipe of *Coprinus sterquilinus* has attained a length of about 10 cm., several drops of the red pigment are excreted from the sides of the pileus, as shown in Fig. 74. The pileus, as it continues to elongate, becomes dull red and finally black. The change in colour to black is chiefly due to the fact that a black pigment is developed in the walls of the spores, but is in part due to the fact that the red cell-sap gradually changes to a dull brown. At the stage shown in Fig. 77 (p. 188) the pileus is so dark that it may be described as black. In its final stages of development, when spore-discharge is well advanced and the process of autodigestion has made considerable progress, the pileus is very black indeed (Fig. 98, p. 234).

FIG. 74.—*Coprinus sterquilinus*. A fruit-body excreting red drops of fluid from its pileus. Just removed from a horse-dung culture covered by a bell-jar. Natural size.

As the stipe elongates, the pileus, which is at first barrel-shaped, becomes conico-cylindrical. During expansion, it first becomes

campanulate (*cf.* Figs. 70, 71, 72, 75), then umbrella-shaped (Figs. 76, 77), and finally almost plane with a more or less revolute rim (Figs. 98, 99, 100, pp. 234 and 236). Its disc usually remains somewhat umbonate to the last. In the expanded condition the pileus is black all over, except for the disc which is tawny and the scattered scales which are usually white although often somewhat pinkish. The dimensions of the mature pileus in well-grown fruit-bodies may be gathered from the illustrations given in Figs. 76 and 77.

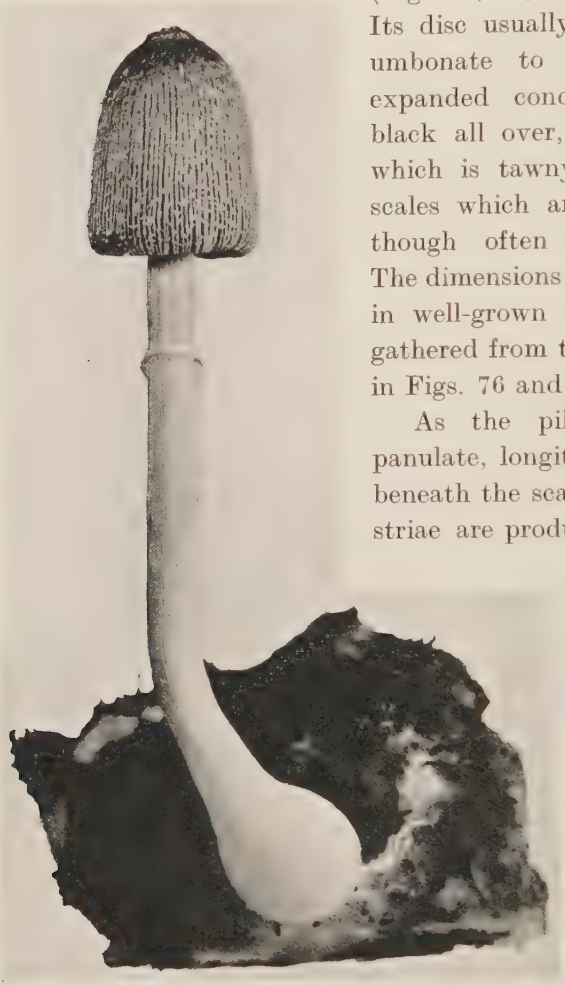


FIG. 75.—*Coprinus sterquilinus*. A fruit-body on sterilised horse dung. The pileus is beginning to expand and, owing to the incipient splitting of each gill into two halves, appears striated. Natural size.

As the pileus becomes campanulate, longitudinal striae appear beneath the scales (Fig. 75). These striae are produced by the incipient splitting of the gills down their backs. As the pileus opens out further, the striæ are converted into sulcations which may become 1 to 2 mm. deep and of the same width at the periphery of the pileus (Figs. 76 and 77). The partial splitting of the gills down their backs, which is shown in a vertical section in Fig. 101, A (p. 237), permits of the rapid expansion of the pileus without any corresponding growth of the pileus-flesh. Occasionally, as the pileus continues to expand, a

few of the gills become entirely divided toward the rim of the pileus into two separate halves. The pileus thus becomes torn into rays (Figs. 98, 99, 100, pp. 234 and 236).

The pileus-flesh, from which the gills are developed in the first place and to which they remain attached during the production and liberation of the spores, consists of a very thin layer external to the gills and of a cap just above the top of the stipe. The thin layer external to the gills becomes split up into radial ribbons during the expansion of the pileus and the partial splitting of the gills down their backs. The flesh which forms the disc of the pileus is sufficiently thick and rigid to provide an apparatus for raising the weight of the sides of the pileus during the period of expansion.

Fig. 76.—*Coprinus sterquilinus.* An expanding pileus. The sulcations are caused by each gill splitting into two halves part of the way down its median plane. The stipe has become tinged with black. Natural size.

The gills, as a rule, are 3 to 6 mm. broad and 2·5 to 5 cm. long. They are at first quite white, then reddish owing to the development of the already-mentioned cell-sap in every cell, and finally purplish-black owing to the browning of the red cell-sap and to the formation of a very dark brown pigment in the walls of the spores. The colour-changes take place from below upwards on each gill (*cf.* Fig. 97, C, p. 232).

The shape of the gills in cross-section is shown in Fig. 78. The two sides of a gill are not quite parallel but are slightly convergent from the flesh to the free margin; but the free margin, instead of ending sharply as in *Psalliota campestris*, *Marasmius*

oreades and the Aequi-hymeniiferae generally, is expanded into a swollen flange. The flanges of the gill-edges of successive gills

Fig. 77.—*Coprinus sterquilinus*. A fruit-body growing on sterilised horse dung in a glass dish. The pileus is expanding and has just begun to shed spores. Each of the sulcations separates the two halves of a single gill. Natural size.

are in contact with one another before the pileus opens and whilst the development of the sterigmata and spores is going on (Fig. 79). The gills are therefore subparallel-sided and flanged. We thus see that, so far as the shape of its gills is concerned,

Coprinus sterquilinus is a typical member of the Comatus Subtype.

The identity in general form shown by the gills of *Coprinus sterquilinus* and *C. comatus* may be realised at a glance by comparing Figs. 78 and 79 in this volume with Plate II, Fig. 5 and Plate III, Fig. 14 in Volume I.

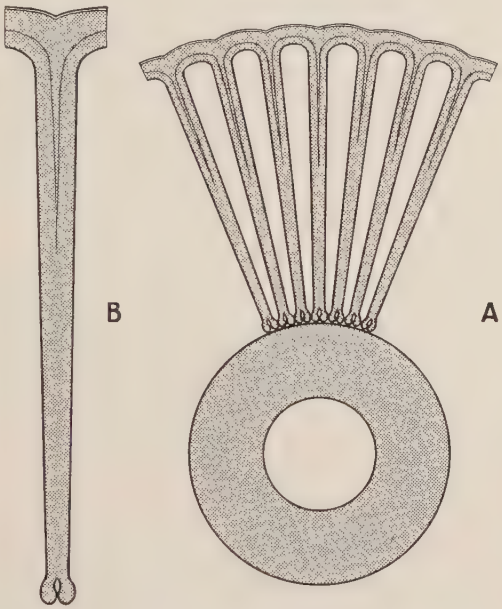


FIG. 78.—*Coprinus sterquilinus.* A, part of a transverse section of a pileus at a stage like that represented in Fig. 71. The hollow stipe and seven gills are shown. Between each pair of adjacent gills there is an interlamellar space. Each gill has a swollen edge or *flange* close to the stipe. B, one of the gills enlarged showing the flange formed by the swollen gill-edge. Magnification: of A, 10; of B, 20.

The significance of the flanged condition of the gills is the same for *Coprinus sterquilinus* as for *C. comatus*, and is to be sought in the nature of the special arrangements provided for the free development of the basidia on the surfaces of opposing gills. Opposing spore-bearing basidia, such as those shown in Fig. 79, must not come into contact with one another or there would be mutual mechanical interference during the production of the spores. Now in *Coprinus sterquilinus* there are no cystidia on the sides of the gills. In *Coprinus atramentarius*, the cystidia which stretch between adjacent gills act as stays or distance-pieces and serve to keep the surfaces of adjacent gills, which are very thin and weak, at such a distance apart as is necessary for the free development of the basidia.[1] In the absence of cystidia, the necessary interlamellar

[1] A. H. R. Buller, "The Function and Fate of the Cystidia of *Coprinus atramentarius*, etc.," *Annals of Botany*, vol. xxiv, 1910, pp. 613–628. Also *vide infra*, chap. ix.

spaces must be developed in some other manner. This is done in *Coprinus sterquilinus* : firstly, by the slight expansion of the pileus so that the gills are suitably spaced where they adjoin the pileus-flesh and, secondly, by the production of flanges where the gills come together in the neighbourhood of the stipe (*cf.* Figs. 78 and 79). Further, the gills are somewhat thicker and correspondingly stiffer than they are in *Coprinus atramentarius* and other species where the cystidia act as space-making organs. Thus, owing to the fact that adjacent gills are separated from one another both near the pileus-flesh and near the stipe, and to the fact that the gills are sufficiently rigid so that sagging does not occur in their central parts, the interlamellar spaces necessary for the free development of the basidia are maintained in existence.

The gills are not all of one length but, just as in *Psalliota campestris* and other Agaricineae with large fruit-bodies, are of various lengths (Fig. 80, B). The longest gills, which in one fruit-body were found to be forty in number, stretch from the periphery of the pileus to near the stipe where they unite and form a *collar* (Fig. 80, *c*). This collar resembles that of *Coprinus plicatilis* and is attached to the flesh of the disc at a radial distance from the stipe of 1–2 mm. The gills of *Coprinus sterquilinus*, therefore, in the language of the field mycologist, are *free from the stipe*. The gill-system is irregular, and it is impossible to separate the gills into natural classes with special limits of length for each class. As two adjacent long gills pass from the collar to the pileus-periphery, they diverge, and, when the divergence has attained a certain degree, a new and shorter gill arises in the interlamellar space between them and proceeds to the pileus-periphery. Other still shorter gills may arise nearer the periphery of the pileus in a similar manner. The number, length, and arrangement of the gills in the gill-system is such that the pileus in the barrel-shaped condition contains just as many gills packed side by side as there is room for, due provision being made for the interlamellar spaces required for the development of the projecting basidia. The development of the gill-system is beautifully regulated, so that the gills are never overcrowded and never too few to occupy fully the space at their disposal.

When the pileus is barrel-shaped, just before its expansion begins, the gills are all compactly crowded into a relatively small space ; but, as the pileus expands, the interlamellar spaces increase

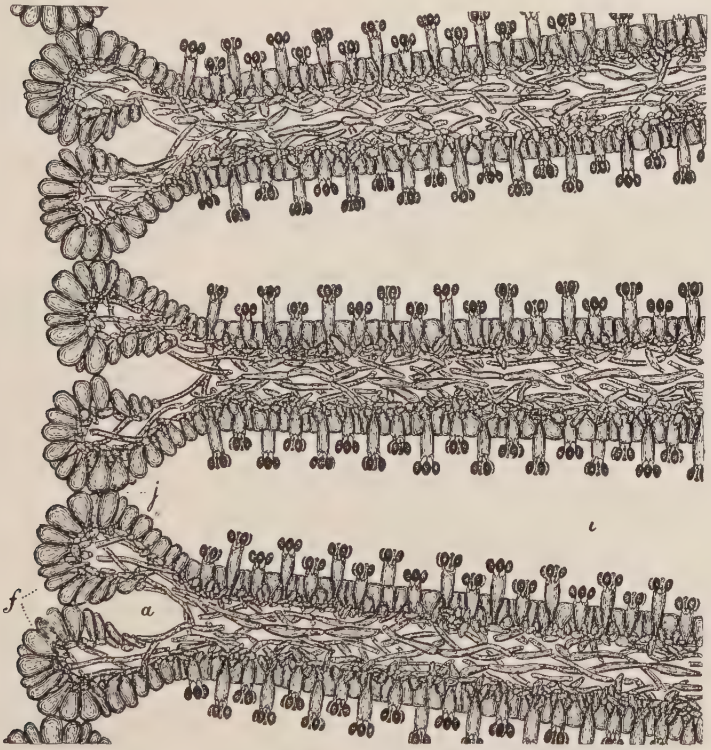


Fig. 79.—*Coprinus sterquilinus*. Parts of three gills of Fig. 78 showing in detail the structure of the swollen gill-edges or flanges and how by the presence of these flanges opposing hymenial surfaces are kept from rubbing against one another. Into the interlamellar space, *i*, the dimorphic basidia are projecting ; *f*, a flange formed by the incurved edges of a gill ; *a*, a large air-space ; *j*, place of junction of two flanges. Cystidia are absent from the gill-sides ; the swollen cells on the exterior of the flange, *f*, are large and sterile. Magnification, 86.

in width, so that finally each gill is freed from its two neighbours and provided laterally with two large interlamellar spaces into which it may discharge its spores without any risk of their striking obstacles. The width of these interlamellar spaces in a fruit-body which has become helmet-shaped is shown in Fig. 101, A (p. 237). In the piece of pileus shown at B in Fig. 80, the number of the gills

which enter into the formation of the collar is 10, whereas the number of gills arriving at the periphery of the pileus is 22. In general, we may say that the total number of gills is slightly larger than double the number of the long gills which form the collar.

The flanged edges of the gills in an unexpanded barrel-shaped

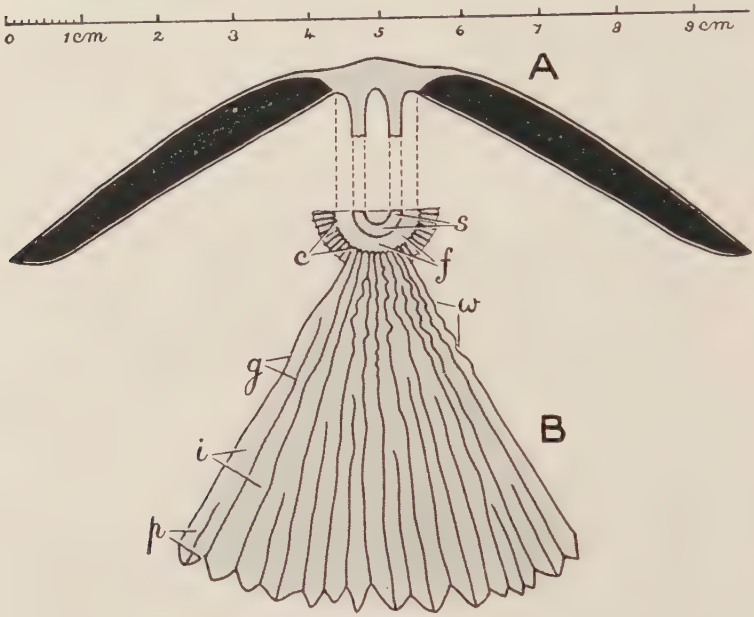


FIG. 80.—*Coprinus sterquilinus*. A, a vertical section through a large pileus which was rapidly expanding and was about to begin to discharge its spores. B, a plan of part of the under side of the same pileus, showing: the gills, *g*; interlamellar spaces, *i*; the stipe, *s*; the naked area of the pileus-flesh, free from gills, *f*; and the collar, *c*, formed by a union of the inner ends of the gills. At the pileus-periphery, *p*, there are 22 gills, but only 10 extend to the collar. The corrugations of the gills, seen at *w*, are being straightened out as the pileus expands. Natural size.

pileus are all locked together so as to make a continuous sheath enclosing the stipe. At first this sheath is quite white; but, subsequently, owing to the development in all its cells of the already-mentioned red cell-sap, it becomes reddish like the other parts of the pileus. It is eventually broken up by the separation of the gills from below upwards and then destroyed by autodigestion from below upwards. If one breaks open a barrel-shaped pileus just before it is about to expand, and if one then examines the sheath in face

view, one usually perceives that the flanged edge of each gill is not straight but wavy (*cf.* Fig. 80, B, *w*). The same phenomenon can be observed in *Coprinus comatus*. This waviness is evidence of the fact that the gills are more or less corrugated toward their flanged edges from below upwards. The lamellae appear to grow faster in length along their flanged edges than where they adjoin the pileus-flesh. Hence the production of transverse corrugations. These corrugations can also be seen when the fruit-body is expanding but, as a rule, they are then less marked. Some of them are shown, particularly toward the upper ends of the gills, in Fig. 81, which represents a pileus which has become helmet-shaped but is not yet flattened. As the pileus gradually expands and flattens out so as to become plane, the lower free margins of the gills are necessarily stretched more than the upper parts attached to the pileus-flesh. The result of this is that the corrugations in the gills, during the flattening of the pileus, become gradually eliminated. The development of the corrugations seems to show that provision for the rapid stretching of the gills in length during the flattening out of the pileus is made in advance. If the gills were not corrugated but simply extended their area

FIG. 81.—*Coprinus sterquilinus*. Under side of the pileus of the fruit-body shown in Fig. 77 (p. 188), just after the beginning of spore-discharge. In the dark peripheral zone, where the spores are being shot out from the gill-edges, the interlamellar spaces are well-developed; but in the lighter central zone, where the white flanges of the gill-edges are still intact, spore-discharge has not yet begun. Natural size.

during the flattening of the pileus, the surface of the gills for producing ripe spores would be less during the spore-producing period than it actually is and, as a consequence, fewer spores would be produced. The transverse corrugations of the gills of *Coprinus sterquilinus* and of *C. comatus*, therefore, must be regarded as a factor, although a minor one, in rendering the fruit-bodies efficient as spore-producing organs. It may be added that the production in young gills of corrugations which are eventually straightened out during the expansion of the pileus is not limited to the two species of Coprini just named, but is found in a number of other Agaricineae. I have noticed it particularly in *Laccaria laccata*.

The gills of *Coprinus sterquilinus*, like those of all other Coprini, are ageotropic. To prove this, all that one requires to do is to fix the opening pileus in such a position that, as it expands, it becomes flattened out not in a horizontal plane but in some other plane inclined at a considerable angle to the vertical. Under such conditions, one observes that the gills do not respond to a geotropic stimulus : they remain in the planes in which they have been placed and do not turn downwards toward the earth. In not being positively geotropic they therefore differ from the gills of *Psalliota campestris* and of other Aequi-hymeniiferae. *Coprinus sterquilinus* would gain nothing by having gills which were positively geotropic, for without this reaction, as we shall see, the spores are discharged in a perfectly successful manner.

A few hours before the spores are to be shed the stipe grows very rapidly in length, for it passes from a stage slightly in advance of that shown in Fig. 97, A (p. 232), to the stage shown in the same figure at C in the course of a single evening. The greatest rate of growth observed in a large fruit-body was 3 cm. in 3 hours, or 1 cm. per hour.[1]

[1] O. Brefeld (*Untersuchungen über Pilze*, Heft III, 1877, p. 61) states that in warm weather the stipe of large fruit-bodies of *Coprinus stercorarius*, during the last half of the stretching period, elongates at a rate of upwards of half an inch an hour. Thus the stipe of *C. stercorarius* may grow in length as fast as, or even faster than, that of *C. sterquilinus*. But the maximum rate of elongation of these stipes—about 1 cm. per hour—is far exceeded by the maximum rate of elongation of certain Phanerogams. Thus the stem of a species of Dendrocalamus—a bamboo—has been observed to grow in length in 24 hours, 57 cm. at Buitenzorg and 91 cm. in a greenhouse at Kew, *i.e.* at rates of 2·4 and 3·8 cm. per hour respectively (*vide* W. Pfeffer *Pflanzenphysiologie*, Leipzig, vol. ii, 1904, p. 17).

The rate of growth in length of the stipe of a less vigorous fruit-body was found to average 0·7 cm. per hour for five successive hours.

The rapid elongation of the stipe during about 12 hours before the beginning of the spore-discharge period does not take place throughout the whole length of the stipe-shaft, but only in that portion which is enclosed within the pileus. This was proved by exact experiment. An elongating fruit-body (Fig. 82, A) was 9·5 cm. high. The exposed part of the stipe below the pileus was 4·5 cm. high, and the barrel-shaped pileus and the enclosed part of the stipe 5 cm. high. A thin vertical slice of the pileus which included about four gills was cut away from the disc downwards, and thereby a portion of the stipe enclosed within the pileus was exposed to view (*s*). Horizontal lines (*i*), made with Indian ink, were then drawn at 1 cm. intervals from the base of the

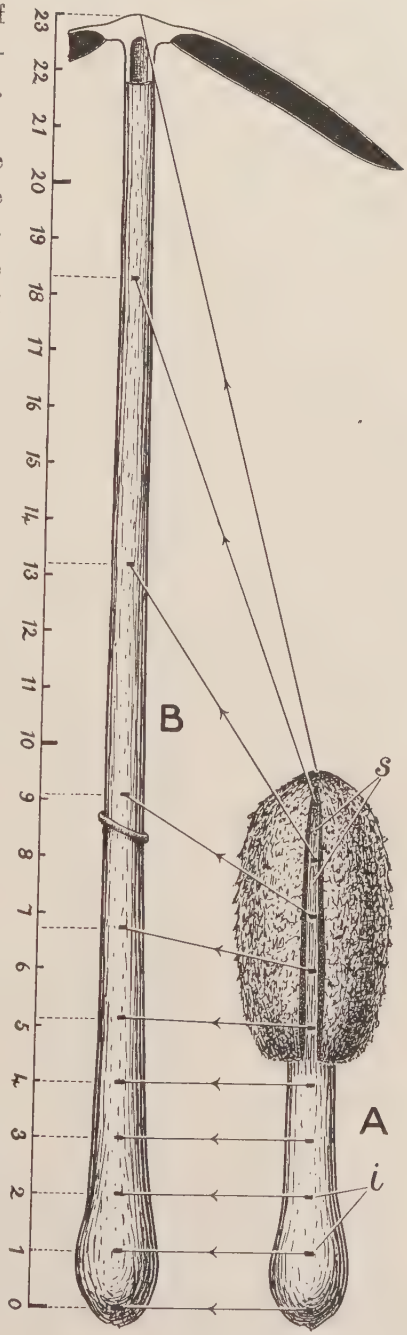


Fig. 82.—*Coprinus sterquilinus*. Elongation of the stipe of a fruit-body growing on horse dung in a laboratory culture. A, a fruit-body from which a piece of the pileus has been removed exposing the stipe *s*. Indian ink marks, *i*, were painted on the stipe from below upwards at intervals of 1 cm. Time 4.45 P.M. B, the same fruit-body next morning at 11 A.M. The stipe has now become 23 cm. long. The change in position of the ink marks is indicated by the arrows. The uppermost part of the stipe has elongated most. The scale is in centimetres. About $\frac{7}{9}$ the natural size.

stipe to its apex. After a night had passed, the stipe was found to have grown from its original length of 9·5 cm. to 23 cm. (Fig. 82, B). It was then observed by reference to the ink marks that that portion of the stipe which had been naturally exposed for a distance of 4·5 cm. below the pileus had not elongated at all, and that the whole of the nocturnal increase in length of the stipe, amounting to 13·5 cm., had been brought about by the growth of that part of the stipe which had been originally enclosed within the pileus. The whole of this originally enclosed part of the stipe had elongated, but the changed positions of the ink marks made it evident that its upper half had grown in length far more than its lower half. The upper half had increased its length from 2·5 cm. to 14 cm., its increment being 11·5 cm., whereas the lower half had increased in length from 2·5 cm. to 4·5 cm., its increment being only 2 cm. The growth in length of the upper half of the originally enclosed piece of stipe had therefore been nearly six times as great as that of the lower half. Fig. 82 (p. 195) indicates graphically the amount of growth of each centimetre of the stipe, and a study of it will show that the amount of growth in length of each centimetre of the originally enclosed part of the stipe varies inversely with its distance from the stipe-apex proceeding from above downwards. In general terms we may say that the greatest amount of growth in an elongating stipe takes place in the apical portion.

The fully extended stipe varies considerably in length according to the vigour of the fruit-body. The shortest stipe I have ever seen was only 8 cm. long, while the longest was 30 cm. The stipes of fruit-bodies which came up spontaneously on horse dung in the laboratory were, as a rule, from 12 to 18 cm. long; but those of fruit-bodies which were raised in pure cultures were usually 18 to 25 cm. long and not infrequently 25 to 30 cm. long. The solid stipe-base is the thickest portion of the stipe: it is usually somewhat swollen or bulbous and may attain a thickness of 1·5 cm. (*cf.* Figs. 70 and 75, pp. 181 and 186). The stipe-shaft into which it passes above is often 1 cm. thick below (Fig. 97, p. 232). It tapers slightly from below upwards, and its thickness just under the pileus becomes reduced to about 0·5 cm. The stipe-shaft has

the form of a hollow cylinder. The hollow space within is of considerable size. Passing down its centre from the disc is a *loose string of hyphae* which is well shown in the photograph reproduced in Fig. 73 (p. 184) but which has been omitted in the drawings in Fig. 97 (p. 232). A similar string of hyphae is to be found in the hollow stipe of *Coprinus comatus*, to which *C. sterquilinus* is so closely related.

At first the stipe is white. Its lower half remains white ; but, whilst elongating and just before the expansion of the pileus, its upper half becomes reddish owing to the development of a red colouring matter in the cell-sap of its outer hyphae. This pigment gradually darkens in colour and becomes very dull brown. The result is that the upper part of the stipe comes to have a blackened appearance. The blackening of the upper part of the stipe which is shown in Figs. 72 (p. 184), 76 (p. 187), and 100 (p. 236) is a normal phenomenon and not due to accident. I emphasise this point because Massee,[1] Rea,[2] and other systematists erroneously state that the stipe turns black on being bruised. I have attempted several times to make the stipe go black by bruising it, but have always failed. There are Agarics which rapidly change colour when bruised, and many such are known among the Boleti ; but *Coprinus sterquilinus* is not one of them.

The shaft of the stipe, partly on account of its hollowness, is fragile : it appears to have its substance reduced to the utmost degree consistent with the carrying out of its supporting function. The extreme base of the shaft and the solid stipe-base are peronate, *i.e.* densely clothed with fine white projecting hyphae. In a small fruit-body which came up spontaneously on horse dung the diameter of the solid part of the stipe-base was found to be 0·9 cm., but the diameter of this solid part plus the hairy coat on its exterior was 1·3 cm.

At the base of the pileus, when this is still barrel-shaped and unexpanded, there is a ring of sterile tissue surrounding the stipe.

[1] G. Massee, "A Revision of the Genus Coprinus." *Annals of Botany*, vol. x, 1896, p. 139.

[2] Carleton Rea, *British Basidiomycetae*, Cambridge, 1922, p. 500.

When the stipe begins to elongate, this ring of tissue may remain attached to the peronate stipe-base, thus forming an *adnate sheath* or *volva* (Figs. 70, p. 181, and 83), or it may be carried upwards on the base of the pileus for some distance and then, as the pileus begins to expand, be set free and dropped upon the stipe so as to form a loose *annulus* (Figs. 72, 74, 75, 82, pp. 184, 185, 186, 195). In the fruit-body shown in Fig. 71 (p. 182) the annulus is still being carried upwards on the base of the pileus, while in that shown in Fig. 97, C (p. 232), it has only lately been dropped upon the stipe. The annulus of *Coprinus comatus* is formed in the same manner as that of *C. sterquilinus*, but in *C. comatus* it never remains below and therefore never forms a volva: it is always carried by the base of the pileus some way up the stipe-shaft and then set free. It may be asked why it is that in *Coprinus sterquilinus* sometimes a volva is formed and sometimes a free ring. It is not unlikely that the answer to this question is to be found in the effect of external conditions: it may be that very moist conditions are favourable to the formation of a looser ring of tissue which may be readily pulled upwards over the stipe so that eventually it becomes converted into an annulus, while drier conditions may result in the formation of a more tightly fitting ring of tissue which cannot be readily pulled upwards over the stipe and which, therefore, when the stipe begins to elongate, must be

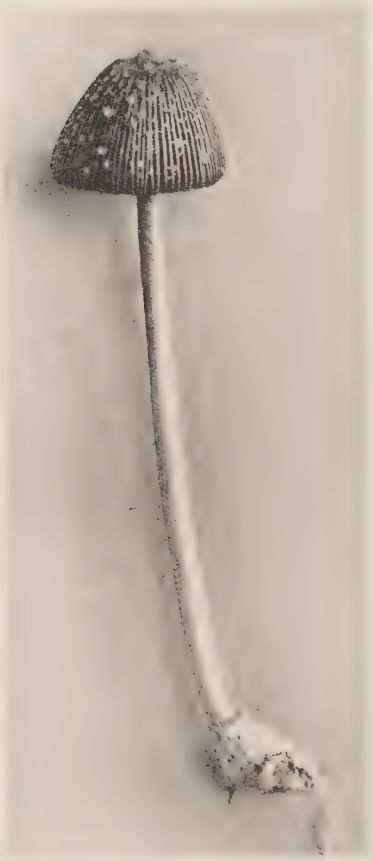


FIG. 83. — *Coprinus sterquilinus*. A very small fruit-body with the annulus left as a volva on the base of the stipe. The pileus has begun to expand. Natural size.

left below to form a volva. However, this suggestion requires to be put to the test of exact experiment.[1]

The Structure and Development of the Hymenium. — The structure of the hymenium of *Coprinus sterquilinus* is similar to that of *Coprinus comatus* which was fully described in the last Chapter. The elements of which the hymenium is composed are basidia and paraphyses only, for cystidia are absent from the sides of the gills. The basidia, as in most Coprini, are dimorphic : they can be divided into two groups—long and short. As in *Coprinus*

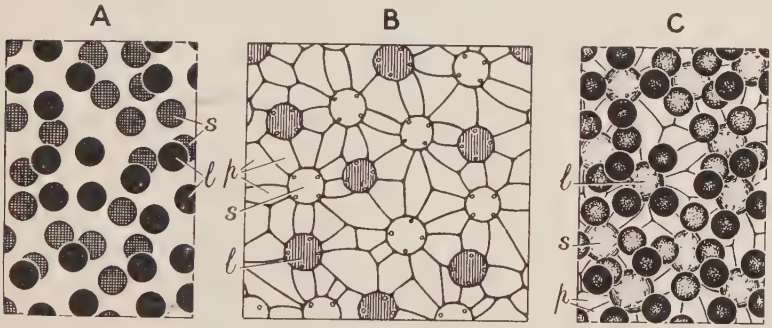


FIG. 84.—*Coprinus sterquilinus*. The hymenium seen in surface view. A, plan of positions of the spores ; *l*, spores of long basidia ; *s*, spores of short basidia. B, plan of positions of long basidia *l*, of short basidia *s*, and of the paraphyses *p*. C, a combination of A and B showing the hymenium with all its elements ; *l*, a long basidium ; *s*, a short basidium ; *p*, paraphyses. The spores of the long basidia often stand partly above the spores of the short basidia. Magnification, 293.

comatus, the number of short basidia on a gill is approximately equal to the number of long ones, but there is a slight preponderance of the former. The long and the short basidia and the paraphyses are so arranged in relation to one another that the hymenium as a whole can be considered as a living mosaic-work with a beautiful pattern.

In Fig. 84 is shown in face view a study of a piece of the hymenium just before the beginning of spore-discharge. The drawing A accurately represents the position of the spores of the

[1] The volva of *Coprinus sterquilinus* is not to be confused with the volva of Amanita, for the former is only an adnate portion of the partial veil whereas the latter is a remnant of the universal veil which at first enwrapped the whole fruit-body. It is the annulus of Amanita which in reality corresponds with the so-called volva of *C. sterquilinus*.

long and the short basidia on a small area. The spores were all equally black in appearance; but, for the sake of clearness, the spores of the long basidia have been uniformly blackened whilst those of the short basidia, which lie at a lower level, have been cross-hatched. In B is given a plan of the arrangement of the basidia and paraphyses after the removal of the spores. The long basidia have been shaded and the short ones left unshaded. It is impossible, owing to optical difficulties, to make a *camera-lucida* drawing which includes the spores as well as the outlines of the basidia and paraphyses. A semi-diagrammatic drawing of this kind, however, is shown at C. It was constructed by adding to the *camera-lucida* drawing A the requisite basidia and paraphyses in a diagrammatic manner, using as a basis the *camera-lucida* drawing B.

As further illustrations of the hymenium when seen in face view we may consider the photomicrographs shown in Figs. 85 and 86. The apparatus with which they were made has already been described in Volume II in connection with an account of a photomicrograph of the hymenium of *Anellaria separata*.[1] However, instead of an arc-lamp, which was not available when required, an electric-light bulb was employed, with the result that the exposure had to be lengthened from about four seconds to four minutes. In making the preparation to be photographed, a piece of a gill bearing fully pigmented spores was dissected from a fruit-body grown in the laboratory and laid flat on a glass slide. A cover-glass was then lightly placed above it to prevent rapid drying. The preparation was therefore mounted in air and not in water. The photomicrograph reproduced in Fig. 85 was taken with the low power of the microscope and has a magnification of 140 diameters. The spores in focus are those of the short basidia, the spores out of focus being those of the long basidia. The tops of the shafts of the long basidia can be seen in places, notably along the upper edge of the photograph toward the right. A study of this photograph will assist the reader in realising that everywhere in the hymenium the long and the short basidia are about equally interspersed, and that there are no cystidia on the sides of the

[1] Vol. ii, 1922, pp. 357–359.

gills. The photomicrograph reproduced in Fig. 86 was taken with the high power of the microscope and has a magnification of 480 diameters. The plane of focus passed through the spores of the long basidia. The spores of the short basidia were below the plane of focus but, nevertheless, here and there, the positions of some

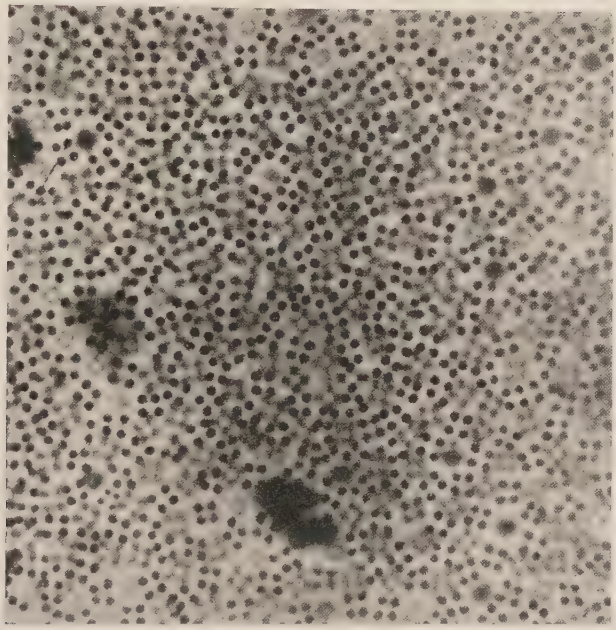


FIG. 85.—*Coprinus sterquilinus.* Photomicrograph of the surface of the hymenium of a gill. The spores of the short basidia are in focus, those of the long basidia out of focus. Magnification, 140.

of them can be dimly perceived. Adjacent long basidia are well separated from one another, so that any lateral jostling of their spores is prevented. Short basidia, which are just as numerous as the long basidia, occupy the spaces between the long basidia, but they cannot be seen owing to their being out of focus.

A cross-section of the hymenium on a smaller scale is shown in Fig. 79 (p. 191) and on a larger one in Fig. 90, D (p. 210), from which it will be seen that, whereas the bodies of the long basidia are very markedly protuberant beyond the general level of the exterior of the paraphyses, the bodies of the short basidia are not

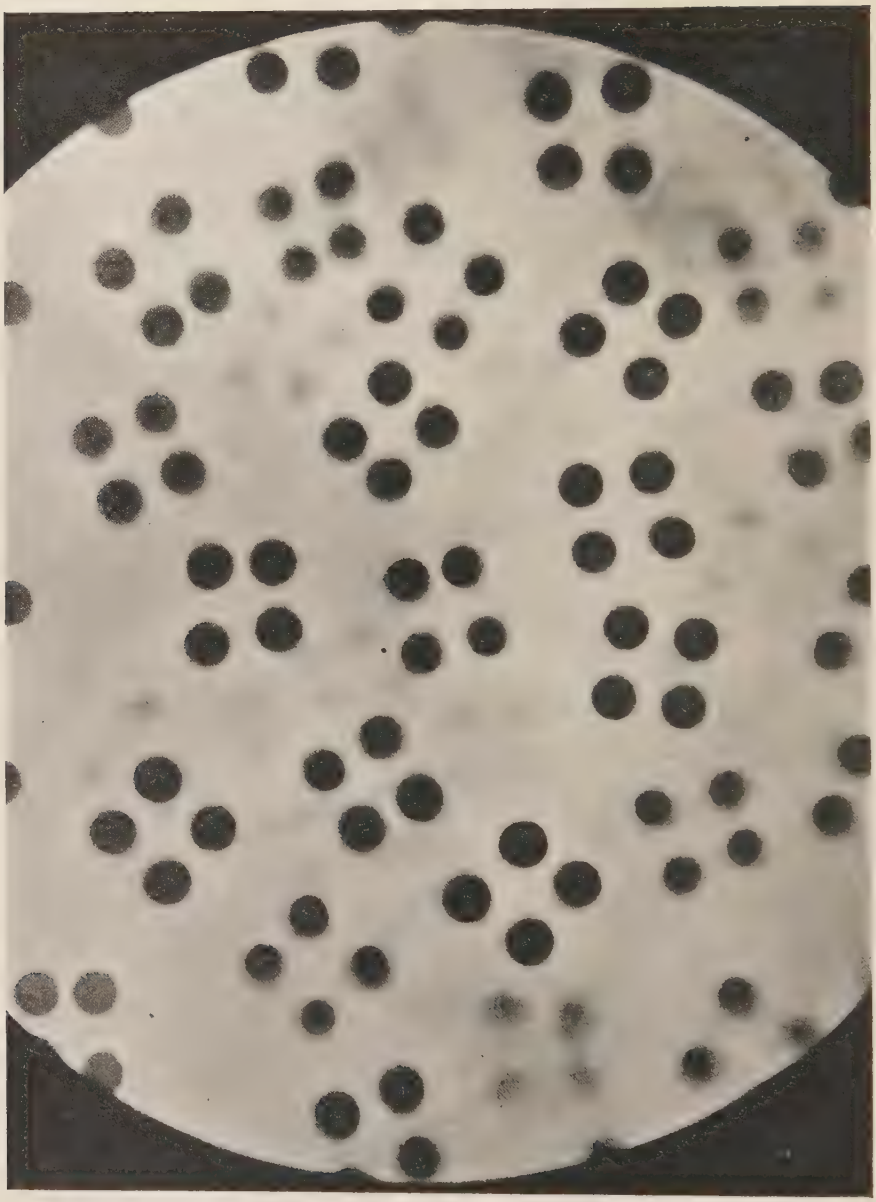


FIG. 86.—*Coprinus sterquilinus*. Photomicrograph of the surface of the hymenium of a gill showing the arrangement of the spores of the long basidia. The spores of the short basidia were below the plane of focus and, consequently, either are invisible or appear only as faint blurs. Magnification, 480.

protuberant at all. The four spores of each basidium are asymmetrically set upon the ends of their sterigmata, so that they stand away from a line drawn through the axis of the basidium-body. They therefore *occupy more lateral space than the basidium-body.* Now all the basidia on a small area of the hymenium, such as is indicated by the cross-section shown in Fig. 90, D (p. 210) or by the surface view shown in Fig. 84, C (p. 199), *must bear ripe or nearly ripe spores at one and the same time* ; for, when the upward-moving zone of spore-discharge comes to envelop such an area, all the spores upon it are discharged within a few minutes. Since adjacent basidia must necessarily bear full-grown spores at one and the same time, and since the four spores on each basidium spread laterally more than do the basidium-bodies, it is evident that adjacent basidia would be at a great disadvantage were their bodies in contact : such contact would result in a mutual jostling of the spores with the result that the development and discharge of the latter would suffer serious mechanical interference. This interference is obviated (1) by the introduction into the hymenium of space-making agents in the form of sterile paraphyses and (2) by the dimorphism of the basidia. The paraphyses surround the body of each basidium so as to isolate it from the bodies of its nearest neighbours. They form a continuous system of elements which are welded together by their lateral walls, and they constitute what may be called a *hymenial pavement* in which at intervals are set the shafts of the basidia. The differentiation of the basidia into two sets, long and short, permits of any small area of the hymenium bearing, at one and the same time, more basidia—and therefore more spores—than would be possible were all the basidia of equal length. In order to realise this the better, let us imagine that the bodies of the short basidia in the section D of Fig. 90 (p. 210) were to be lengthened so that they came to be of the same length as those of the long basidia. At once, some of the spores of the previously short basidia would touch some of the spores of the previously long basidia. The extent and frequency of the jostling of the spores which would result, were the short basidia as long as the long ones, will become even clearer, if we imagine the elongation of the bodies of the short basidia to take place in such a piece

of hymenium as is represented in Fig. 84, C (p. 199). We may therefore conclude that the introduction of the phenomenon of basidial dimorphism into the structure of the hymenium is a distinct factor in fruit-body efficiency : it allows more spores to be produced on each gill than would otherwise be possible.[1]

We shall now discuss the manner in which the hymenium develops. This matter is not only interesting in itself, but appears to throw light upon the origin of the Coprinus Type of organisation from the Non-Coprinus Type as represented by *Panaeolus campanulatus*.

The gills of *Coprinus sterquilinus* change their colour in accordance with the rule which is common for all species of the genus Coprinus : they become blackened from below upwards (Fig. 97, B, C, D, p. 232). This blackening is an expression of the fact that the hymenium on each gill is subjected to a wave of development which progresses upwards in a zonewise manner. The first basidia to begin to develop spores are those which are situated along the bottom of each gill, and the last basidia to begin to develop spores are those which are situated at the top of each gill ; and, since the basidia all take about the same number of hours to develop and ripen their spores, we find that the first basidia to ripen their spores are those which are situated along the bottom of each gill and that the last basidia to ripen their spores are those which are situated at the top of each gill. In other words : on every gill, the spores begin their development and attain maturity in succession from below upwards. As the spores ripen, a black pigment is developed in their walls, and it is this black pigment which is chiefly responsible for the blackening of the gills from below upwards in the course of their development.[2]

Some hours before the stipe begins to elongate rapidly, the gills within the barrel-shaped pileus are not yet fully extended and are still perfectly white (Fig. 97, A, p. 232) ; but the hymenium covering

[1] For a still more detailed analysis of the phenomenon of dimorphism, the reader is referred to the previous Chapter on *Coprinus comatus*, pp. 152–155.

[2] The red cell-sap, which is developed in all the cells of each gill and which gradually turns dull brown, is also a factor, although relatively a minor one, in the darkening of the gills from below upwards. This matter was fully dealt with in the preceding Section, pp. 185, 187.

their sides is undergoing important developmental changes. If examined in face view at the time indicated, the hymenium has the appearance shown in Fig. 87 at A. At this stage of development the paraphyses are still very small and rudimentary, but the basidia are well advanced. The bodies of the basidia have already attained their full growth both in width and length, but as yet they bear no spores and have not even begun to develop their sterigmata. Careful focussing up and down reveals the fact that the basidia can be divided into their two classes, long and short, so that it is evident that the pattern of the hymenial mosaic-work has been already established. The bodies of the basidia develop to their maximum thickness, and therefore attain their maximum lateral extension, much sooner than do the paraphyses. This fact is brought out in a striking manner by comparing the area of the hymenium which has just been described (Fig. 87, A) with an area of equal size obtained from the same fruit-body 36 hours later (Fig. 87, B). Such a comparison shows that, whereas the diameters of the basidia have remained unaltered, the areas of the outer surface of the paraphyses have been increased to some three or four times what they were originally. In the drawings of Fig. 87, for the sake of distinction, the long basidia have been represented in uniform black and the short basidia have been shaded; but there are no real colour-differences between the long and the short basidia when seen with the microscope.

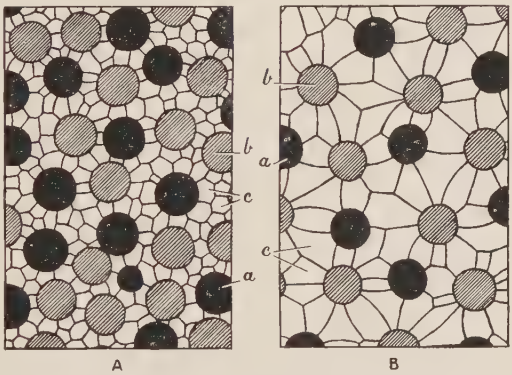


Fig. 87.—*Coprinus sterquilinus.* Development of paraphyses. A, plan of surface view of very young hymenium before the formation of spores. The basidia have attained their maximum diameter but the paraphyses are still rudimentary; *a*, a long basidium; *b*, a short basidium; *c*, paraphyses. B, plan of fully extended hymenium after the spores have been developed; *a*, a long basidium; *b*, a short basidium; *c*, paraphyses. The paraphyses have become greatly swollen, so that there are now only 16 basidia in the area instead of the 27 shown in A. Magnification, 287.

The enlargement of the paraphyses from the size shown in Fig. 87, A, to that shown in Fig. 87, B, takes place whilst the spores are developing on their sterigmata and the gills are growing in length and breadth (*cf.* Fig. 90, A–D). There can be no doubt that the paraphyses, by increasing in size, are responsible for the stretching of the hymenium as the pileus expands and the gills turn away from the stipe, and they may therefore be thought of, in contradistinction to the basidia, as the elastic elements of the hymenium.

A cross-section of the hymenium corresponding to the surface view of Fig. 87, A, is shown in Fig. 89 at A (p. 208). A study of this section makes it evident that the long basidia, the short basidia, and the paraphyses are all clearly differentiated from one another, and further, that each of the basidia contains a single nucleus. The nuclei have a diameter of about $8{\cdot}5\ \mu$ and are so large that they can be seen without difficulty in the living basidia mounted in water or in dead basidia treated with iodine. To the subject of nuclei we shall return later on. The basidium-bodies, when very rudimentary, do not contain any glycogen but, when just about to produce sterigmata, glycogen particles appear at their distal ends, as may be proved by the red-brown reaction with iodine (Fig. 89, A, *g*, p. 208). The glycogen particles appear in the long basidia before they appear in the short basidia ; and this is one of the signs that the former begin to mature sooner than the latter.

The further development of the hymenium was followed by studying cross-sections taken through the living gills. Since, in a single gill, the basidia ripen their spores from below upwards, it is impossible to determine the rate of hymenial development by cutting sections of a single gill at higher and higher levels of the barrel-shaped pileus at successive intervals of time. To determine the rate of hymenial development and, at the same time, to observe a series of successive developmental stages in the hymenium, including the stage shown in Fig. 89, A, a series of sections was therefore taken at successive intervals of time, not at higher and higher levels from a single gill, but at the same level from a series of gills, fresh and previously uninjured gills being used for each successive observation. The details of the method employed for these observations may be described as follows. A fruit-body

was grown in the laboratory on horse dung under a bell-jar. In course of time its pileus became barrel-shaped and attained a stage of development which experience had taught me normally precedes the spore-discharge period by about 36 hours (*cf.* Fig. 70, p. 181). A small block of tissue (Fig. 88, A), about 6 mm. high, just sufficiently large to be held comfortably in the fingers for section-cutting with a razor, was thereupon removed from the base of the pileus with the help of a sharp scalpel, care being taken to do as little damage as possible to the fruit-body so that its development might continue in a normal manner. Sections were then made with a hand-razor from the upper surface of the block of tissue, several gills being cut through transversely at a distance of 5–6 mm. above their extreme lowest points at the basal rim of the pileus. Thereafter, at the same height above the rim of the pileus and at successive intervals of time, similar sections were taken from a series of blocks of tissue (B, C, etc., in Fig. 88) cut away from the base of the pileus in a similar manner to the first block. The sections taken from all the blocks were compared and thus the rate and mode of development of the hymenium was worked out. It will thus be seen that the method of obtaining sections and making comparative observations from them, as just described, is based on the fact that all the gills develop in an equal manner, in that a single wave of hymenial development passes upwards on all the gills simultaneously from the base of the barrel-shaped pileus to its apex. The sections were all mounted in water and studied in the living condition immediately after they had been cut.

FIG. 88.—*Coprinus sterquilinus.* A young fruit-body just before the development of sterigmata and spores. A, B, C, etc., blocks of tissue which were removed at successive intervals of time and from the tops of which transverse sections were cut for the purpose of studying the rate of development of the sterigmata and spores. Natural size.

The section sketched in Fig. 89, A, was made from a block of material removed from the pileus at 5 P.M. As already pointed out, it shows that the long basidia, the short basidia, and the paraphyses are all clearly differentiated from one another ; but that the basidia

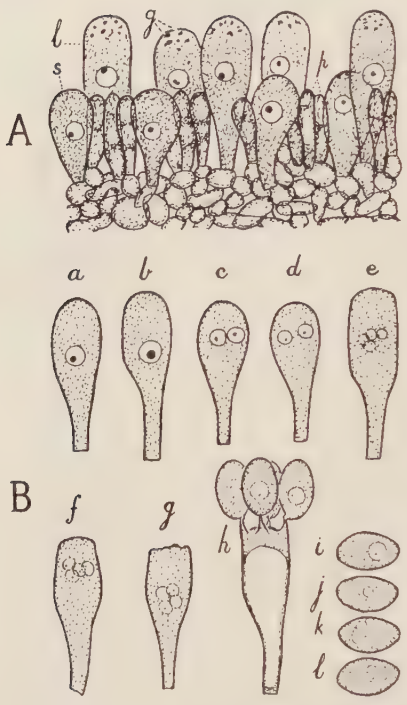


FIG. 89.—*Coprinus sterquilinus.* Nuclei in living basidia. A, cross-section through a living hymenium showing a single nucleus with a nucleolus in all the basidia; *l*, long basidium; *s*, short basidium; *p*, paraphyses; *g* glycogen particles (staining brown with iodine) in all the long basidia. B, basidia showing nuclear changes; *a* and *b*, with one nucleus each; *c* and *d*, with two nuclei each; *e*, *f*, and *g*, with four nuclei each; *g* shows the beginning of sterigmata; *h*, a basidium with four spores—the nuclei have crept up into the spores into which also the cytoplasm is passing, so that the body of the basidium contains a large vacuole; *i*, an isolated spore containing one nucleus; *j* and *k*, two spores each containing two nuclei; *l*, spore with four more or less definite clear spaces in the centre which are possibly nuclei. Magnification, 352.

are only represented by their bodies and contain no more than a single nucleus (the fusion-nucleus), while the paraphyses are still extremely slender. At 10 P.M., a section taken at the same level from another block of material exhibited the appearance represented in Fig. 90, A. During the preceding five hours, sterigmata had developed upon the ends of the long basidia. Moreover, nuclear division had taken place. The stages in the division of the nucleus were studied in sections taken between 5 P.M. and 10 P.M.; and some of them are illustrated in Fig. 89, B. Up to 6 P.M. the long basidia each had but one nucleus (*a* and *b*) and were therefore similar to the long basidia shown in Fig. 89, A. By 8.30 P.M., however, some of them already possessed four nuclei (*e*, *f*, and *g*), the intermediate binucleate condition (similar to that shown for two short basidia at *c* and *d*) having been passed through in the preceding two and a half hours. Thus the division of the original fusion-nucleus first into two smaller nuclei and then into four still smaller nuclei was accomplished in less than three hours. Possibly in a single basidium the two successive bipartitions of the original nucleus take place in about an hour and a

half, but the exact duration of the dividing period cannot be given owing to the impossibility of watching one and the same basidium continuously as it undergoes its development. It was found that, after a transverse section of a gill had been immersed in water, the nuclei of the basidia failed to undergo any further changes and that in the course of an hour or two the basidia lost their vitality. The basidia became flattened at their ends and showed the first traces of sterigmata just after the second nuclear bipartition (*f* and *g*). The nuclear changes observed in the long basidia repeated themselves in the short basidia. Several short basidia were found to be in the binucleate stage at a time when the adjacent long basidia were in the quadrinucleate stage and, in general, it was observed that the nuclear changes of the short basidia lagged behind those of the long basidia. As soon as the sterigmata became developed, it was found impossible to detect any nuclei whatever in any of the basidia.

Various forms of the hymenial elements, as seen at successive intervals of time, are shown in Fig. 90. The sketch A was made at 10 P.M., the sketch B at 1 A.M., and the sketch C at 2.30 A.M., whilst the sketch D represents the condition of the hymenium at about 12 midnight. If therefore we take 5 P.M., at which time the sketch reproduced in Fig. 89, A (p. 208) was made, as the zero of a time-scale, then the sketch A in Fig. 90 represents a stage of development after an interval of 5 hours, sketch B a stage after 8 hours, sketch C a stage after 9·5 hours, and sketch D a stage after about 31 hours.

If the sketches A, B, and C in Fig. 90 are carefully observed, it will be noticed that in each of them the long basidia are somewhat further advanced in their development than the short basidia : for in A the long basidia have their sterigmata developed before these structures have begun to appear upon the short basidia ; in B the long basidia have already commenced to develop spores, whilst the short basidia as yet merely possess sterigmata ; and in C the spores of the long basidia are half-grown, whilst those of the short basidia are nothing more than minute rudiments. In D, which represents the appearance of the hymenium just prior to the discharge of the spores, the spores of the long and the short basidia are equally

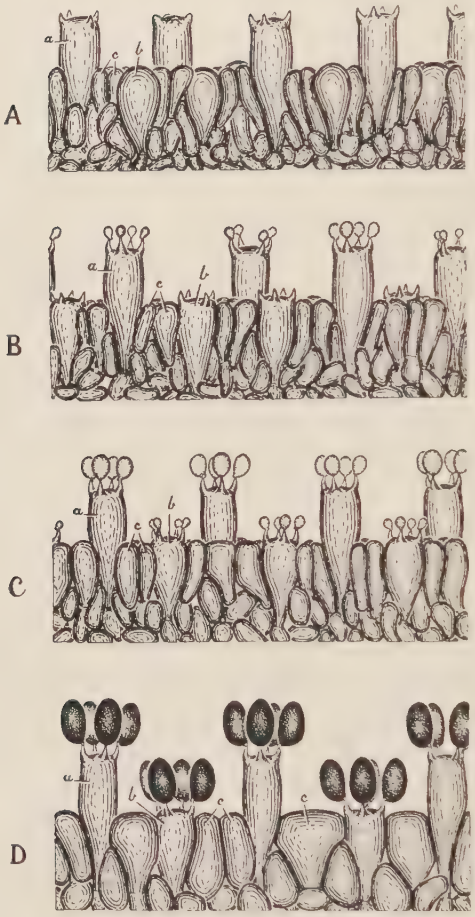


Fig. 90.—*Coprinus sterquilinus.* Development of hymenium as seen in cross-section, providing evidence that there are two generations of basidia. A, at 10 P.M., the long basidia, *a*, have developed sterigmata, the short basidia, *b*, have not; *c*, the rudimentary paraphyses. B, at 1 A.M., the long basidia, *a*, are developing spores; the short, *b*, have only developed sterigmata; *c*, the paraphyses. C, at 2.30 P.M., the long basidia have larger spores than the short ones; *c*, the swelling paraphyses. D, at 12 midnight, both generations of basidia have full-sized black spores, ready for discharge; *c*, the swollen paraphyses. Magnification, 287.

sterquilinus with that of *Panaeolus* large and equally black. But between the stages C and D intermediate stages were observed. In one of them it was seen that the spores of both the long and the short basidia had attained full size, but that the spores of the long basidia had commenced to develop pigment in their walls, whereas the spores of the short basidia were as yet quite colourless.

It seems to me that the study of the development of the hymenium as described above, in which it was shown that the long basidia lead the short basidia (1) in the division of the nucleus, (2) in the development of sterigmata, (3) in the development of spores, and (4) in the development of pigment in the spore-walls, warrants us in drawing a conclusion of considerable importance, namely, that *the long basidia all belong to a first generation of basidia, and the short basidia to a second generation.* If we compare the development of the hymenium of *Coprinus campanulatus,* which was

given in detail in Volume II,[1] it seems clear that we can regard the two successive generations of basidia which arise in the former species as comparable with the first two of the numerous successive generations which arise in the latter species. Since the structure and development of the hymenium of most species of Coprinus are similar to those of *Coprinus sterquilinus*, we may say, speaking generally, that *in the Coprini the number of generations of basidia has become reduced to two* and that the development of the second generation takes place earlier than it does in such a non-Coprinus fungus as *Panaeolus campanulatus*. In the Panaeolus Sub-type, spores do not begin to develop on the sterigmata of a succeeding basidial generation until those on the sterigmata of the preceding basidial generation in the immediate neighbourhood have been discharged. In the Coprini, however, this is not the case, for in them the development of the spores of the second-generation basidia is hastened, so that, shortly after the basidia of the first generation have begun to develop spores, the basidia of the second generation begin to develop spores also; and this overlap in the development of the two basidial generations is of such a nature that the second-generation basidia develop somewhat faster than the first-generation basidia, with the result that, in the end, just before spore-discharge takes place, on any small hymenial area (0·1 mm. square, *cf.* Fig. 90, D), not merely all the basidia of the first generation, but also all those of the second, bear spores which are full-grown, perfectly ripe, and ready for projection from the sterigmata. The comparison of hymenia which has just been made and which has an important bearing upon the question of the evolution of the Coprinus Type may perhaps be still more fully realised by comparing Fig. 90, D (p. 210) in this volume with Fig. 98, A (p. 292) in Volume II.

A few more facts still remain to be recorded concerning the behaviour of the nuclei. The spores, whilst growing from tiny rudiments to full size, are unprovided with nuclei; but, soon after they have attained full size, a nucleus appears in each, as is shown in Fig. 89 at *h* (p. 208), which represents a living basidium seen in water. By using appropriate staining reagents applied to

[1] Vol. ii, 1922, pp. 264–297.

microtome sections, the four nuclei of certain basidia were observed, as it were, in the act of entering the bases of the sterigmata ; and there can be no doubt that, as in other Hymenomycetes, the four nuclei of each basidium-body make their way through the extremely narrow sterigmatic passages into the spores. A nucleus, after creeping into a spore, rounds itself off (Fig. 89, *h*) and soon divides into two (*j* and *k*). Two nuclei were observed several times in living spores immersed in water and also in dead spores which had been stained. Sometimes, in a living spore, four rounded bodies could be made out (*l*), but I was unable to decide whether they were nuclei or merely vacuoles.

An important phase of the development of the four spores of a basidium is the passage into them of the contents of the basidium-body. Before a basidium develops its spores it is usually loaded with protoplasm, and no prominent vacuole is to be seen within it. With the development of the spores and their growth to full size, a vacuole makes its appearance toward the base of the basidium-body and gradually extends its boundary in the direction of the spores until, eventually, it comes to occupy the whole of the interior of the basidium-body with the exception of the tip of each sterigma. Its wall is a very thin layer of cytoplasm which is closely appressed to the cell-wall. The existence of this layer of cytoplasm must be assumed, for only by its presence could the osmotic pressure of the cell-sap, upon which the turgidity of the basidium-body depends, be maintained during the ripening and discharge of the spores. An advanced stage in the development of the vacuole is shown at *h* in Fig. 89 (p. 208). The growth of the vacuole is an indication that the contents of the basidium-body are passing upwards into the spores. The emptying of the basidium-body and the corresponding filling of the four spores takes several hours for its completion. The basidium is evidently a *spore-mother-cell* in that its dominating function is to produce and discharge spores. It yields practically all its contents to its four spores, thus providing them with protoplasm which is rich in food materials and suited to the production of germ-tubes and rudimentary mycelia. There is evidently a direct relation between the capacity of a basidium-body and the collective capacity of the four spores which it produces.

If we compare the basidia of Hymenomycetes generally, we are safe in laying down the rule that *the capacity of the four spores of a basidium of any species is directly proportional to the capacity of the basidium-body.* But, other things being equal, the dimensions (length, breadth, and thickness) of a cell vary with its capacity. From this we may draw the conclusion that the dimensions of the spores in Hymenomycetes are proportional to the dimensions of the basidia which bear them, or, in other words, *the size of the spore and the size of the basidium in any species are correlated with one another.*

One may ask: what causes the cytoplasm and the nuclei of the basidium-body to pass upwards through the exceedingly narrow sterigmatic channels into the spores? It is now well-known, owing to the investigations of cytologists: that the four nuclei of the basidium-body are at first spherical; that, as they pass through the sterigmata, they become moulded by the pressure of the sterigmatic walls into slender conical threads; and that, as soon as they have emerged into the spores, they at once resume their spherical form. When, in a stained microtome section, one observes one of these nuclei in a slenderly conical sterigmatic cavity, one may feel inclined to think that, when living, it was creeping through the sterigma in an amoeboid manner or that it was being drawn through the sterigma by the contraction of a kinoplasmic thread connecting the nuclear membrane with the apex of the spore;[1] but there is another possible hypothesis—first suggested to me by Dr. Harold Wager—which appears to explain in a very simple and satisfactory manner not merely the passage of the nuclei through the sterigmata but also the passage of the cytoplasm. This hypothesis is as follows. When the cytoplasm and the nuclei in a basidium-body begin to pass upwards through the four sterigmata into the spores, a vacuole, as we have seen, always forms at the base of the basidium-body and then grows in size *pari passu* with the emigration of the basidium-body's protoplasmic contents. Moreover, the boundary surface between the vacuole and the disappearing protoplasm is always curved in such a way as to

[1] Concerning the existence of the thread and its significance, *vide* vol. ii, 1922, pp. 419–421.

indicate that the cell-sap of the vacuole is exercising a considerable hydrostatic pressure upon the cytoplasm which contains it (*cf.* Fig. 89, B, *h*, p. 208). It therefore seems highly probable that the principal factor in causing the flow of the cytoplasm and the nuclei of the basidium-body into the four spores is the hydrostatic pressure exerted upon them by the cell-sap of the basidium-body vacuole.

During the development of the fruit-body of any of the Agaricineae, the chemical substances present in the mycelium are transferred to the stipe, the pileus, the gills, and the spores, where they are made use of in various ways. This transference, no doubt, is a very complex process and involves numerous chemical transformations of substances such as proteins, carbohydrates, etc. A complete study of these transformations for any species yet remains to be accomplished. Chemists who desire to study the chemical changes taking place in fruit-bodies ought to bear in mind that the main function of a fruit-body is to produce and liberate hundreds or even thousands of millions of food-laden spores and that, since the spores are liberated in a continuous stream from the under side of the pileus hour after hour often for several days or sometimes weeks in succession, the fruit-body of necessity must become poorer and poorer in food substances. The spores should be collected in the form of a spore-deposit and their contents carefully analysed. By proceeding in this way, one might determine the amount of the original contents of the mycelium or fruit-body carried off by the spores and, at the same time, throw light upon the fate of such carbohydrates as glycogen, trehalose, mannite, and glucose which are often present in the pileus in considerable quantities.

A chemical analysis of the fruit-body of *Coprinus sterquilinus* from the point of view of the production and liberation of spores has not yet been carried out. However, a few observations have been made upon the appearance and disappearance of glycogen, and these may be here recorded. The presence of glycogen was detected with iodine which, as we know from the work of Errera and others, turns glycogen reddish-brown.[1] The gills examined

[1] *Cf.* J. Zellner, *Chemie der Höheren Pilze*, Leipzig, 1907, p. 115.

were removed from a fruit-body growing normally in the laboratory and were tested immediately after their removal. On testing the hymenium in a very young stage of its development, when the basidia possessed only bodies but no sterigmata or spores, no trace of glycogen could be found in it: the basidium-bodies and the paraphyses stained yellow with iodine, thus revealing the presence of proteins only. However, in the same preparations there was an abundance of glycogen in the cells of the subhymenium and trama. At a little later stage in the development of the basidia, when the nuclei were about to divide, clumps of glycogen began to appear at the ends of the basidium-bodies, as shown at *g* in Fig. 89, A (p. 208). Still later, the basidium-bodies became laden with glycogen and showed the reddish-brown reaction with iodine in a very marked degree. At this stage the reddish-brown colour was exhibited by the contents of all the cells of the trama, subhymenium, and hymenium, including the paraphyses and basidia. Shortly after attaining full size and while still possessing colourless walls, the spores on the sterigmata stained intensely reddish-brown with iodine, and it was therefore evident that the glycogen in the basidium-bodies was being transferred to the spores. On becoming older, the spores gradually lost their glycogen contents. At first the glycogen was evenly spread throughout the spore-protoplasm; but, as it was disappearing, it separated into clumps. These clumps became smaller and smaller until, finally, the iodine yielded no trace of them. It thus appears that, in *Coprinus sterquilinus*, as in other Hymenomycetes and in the Ascomycetes, glycogen is not stored up as a reserve food material in the spores. What becomes of the glycogen in the spores whilst it is disappearing? It must be transformed into some other chemical substance, possibly another carbohydrate such as trehalose,[1] and this is doubtless packed away in the spore so that it may lie in reserve and be drawn upon at the time the germ-tube is developed. An analysis of the contents of ripe spores might solve this problem. From what substance, in the first place, is the glycogen in the gills formed? Possibly from a fat [2] but, as the facts of chemical analysis are not at our disposal, this supposition is merely speculative.

[1] Cf. Zellner, *op. cit.*, pp. 116–117.

[2] *Ibid.*, p. 117.

A series of successive developmental stages of long basidia situated about 6 mm. from the rim of a single pileus is shown in Fig. 91.

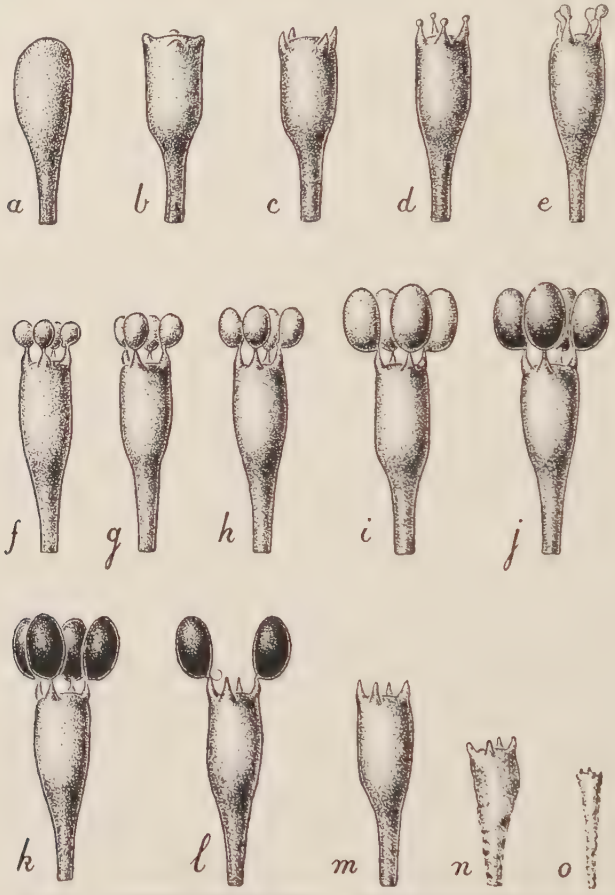


FIG. 91.— *Coprinus sterquilinus*. Stages in the development of a long basidium; *a* at 8 P.M.; *b* at 9 P.M.; *c* at 10 P.M.; *d* at 11 P.M.; *e* at 12 midnight; *f* at 2 A.M.; *g* at 2.30 A.M.; *h* at 3 A.M.; *i* at 8–11 A.M.; *j* at 7 P.M., spores turning brown; *k* at 12 midnight to 6 A.M., spores black; *l* about 6 A.M., two spores have been discharged and a third with a water-drop at its base is about to be discharged; *m* at 6.10 A.M.; *n* at about 6.40 A.M., undergoing autodigestion; *o* at about 7 A.M. just before entire destruction. All the stages from *a* to *o* were passed through in thirty-six hours. Magnification, 408.

Stage *a* was sketched at 8 P.M., stage *b* at 9 P.M., and stage *c* at 10 P.M. The development of the sterigmata therefore took about two hours. Stage *d* was sketched at 11 P.M., stage *e* at 12 midnight, stage *f*

at 2 A.M., stage *g* at 2.30 A.M., stage *h* at 3 A.M., and stage *i* at about 8 A.M. Hence it is clear that the growth of the spores from their first tiny rudiments on the top of the sterigmata to full size is a long process which takes about nine hours for its accomplishment. For several hours (at least three) after a spore has attained its full size, it remains colourless, and then its inner wall slowly becomes pigmented. Stage *j* which shows the spores turning brown was sketched at 7 P.M., *i.e.* eleven hours after the stage *i*, and stage *k* which shows the spores fully pigmented was sketched at 12 midnight. My observations indicate that a spore, after attaining full size, takes about twelve hours before it becomes fully pigmented. The stage *k* persisted from 12 midnight to about 6 A.M., at which time spore-discharge began. At the stage *l*, which represents the condition of a basidium at about 6 A.M., two spores have been discharged and a third is about to be discharged. The spore about to be discharged is the one which has just excreted a drop of fluid at its hilum ; for here, as in all other Hymenomycetes,[1] the appearance of the drop is a signal that the moment of discharge is to be expected within a very few seconds. At the stage *m*, representing a basidium at about 6.10 A.M., the last of the four spores has just been discharged, but the basidium-body is still turgid. At the stage *n*, representing a basidium at about 6.40 A.M., the basidium-body has collapsed and is undergoing autodigestion ; and, finally, at the stage *o*, representing a basidium at about 7 A.M., the whole basidium has become reduced to but a shadow of its former self and is rapidly becoming disintegrated.

The various observations which have been made upon the development of a long basidium may be now summarised as follows. The division of the fusion-nucleus into four nuclei takes from two to three hours. The sterigmata begin to develop very shortly after the last of the two nuclear divisions in the basidium-body has been accomplished ; they complete their development in about two and a half hours ; and, as soon as they have attained their full length, they begin to develop spores at their free ends. The growth of the spores from tiny rudiments to full size takes about nine hours. The time required for the development of the spores

[1] *Cf.* vol. ii, 1922, pp. 8–9.

from full size, to the fully pigmented condition is at least twelve hours; and, after the spores have become fully pigmented, another twelve hours may elapse before spore-discharge takes place. The duration of the period from the beginning of the division of the fusion-nucleus to the discharge of the last spore is about thirty-six hours.

The average length of time taken for the development and ripening of an individual spore from the moment the spore appears on the sterigma as a tiny rudiment until the moment of discharge is a little less than one hour in *Collybia velutipes* and *Dacryomyces deliquescens*; between one hour and one hour and thirty-one minutes in *Collybia fusipes*, *Marasmius oreades*, *Exidia albida*, *Calocera cornea*, *Armillaria mellea*, and *Collybia radicata*; and between five and eight hours in *Stropharia semiglobata*, *Psalliota campestris*, and *Panaeolus campanulatus*; but in *Coprinus sterquilinus* it is about thirty-two hours.[1] The relatively great length of the time required by our Coprinus for spore-development and spore-discharge appears to be due in part to the very large size of the spores, but largely to the fact that all the basidia on any small area of the hymenium develop simultaneously instead of in successive groups. It is also not improbable that the spores on any part of a gill are completely ripened and prepared for discharge some minutes or possibly hours before the upward-moving zone of spore-discharge brings with it the stimulus for discharge, provision thus being made for a margin of safety comparable with that most people allow when they go to a railway station to catch a train.

The Spores.—The spores of *Coprinus sterquilinus* are circular in outline when seen from above (Fig. 86, p. 202), which shows that their breadth and thickness are equal. They are much longer than they are broad or thick, for they are oval in outline (Fig. 92) and measure 20–22 by 11–12 μ.[2] They are by far the largest spores

[1] Vol. ii, 1922, pp. 44, 54.

[2] These measurements were made from the spores of a single fruit-body. Mr. W. F. Hanna, working in my laboratory, has measured the length of 100 spores of a series of fruit-bodies of *Coprinus sterquilinus*, each derived from a monosporous culture, and has found that the average length of the spores varies in different fruit-bodies from about 15 μ to 22 μ. A full report of this work will be published shortly.

of any hymenomycetous fungus which I have as yet investigated. A single spore is relatively so gigantic that, as indicated in Fig. 93,

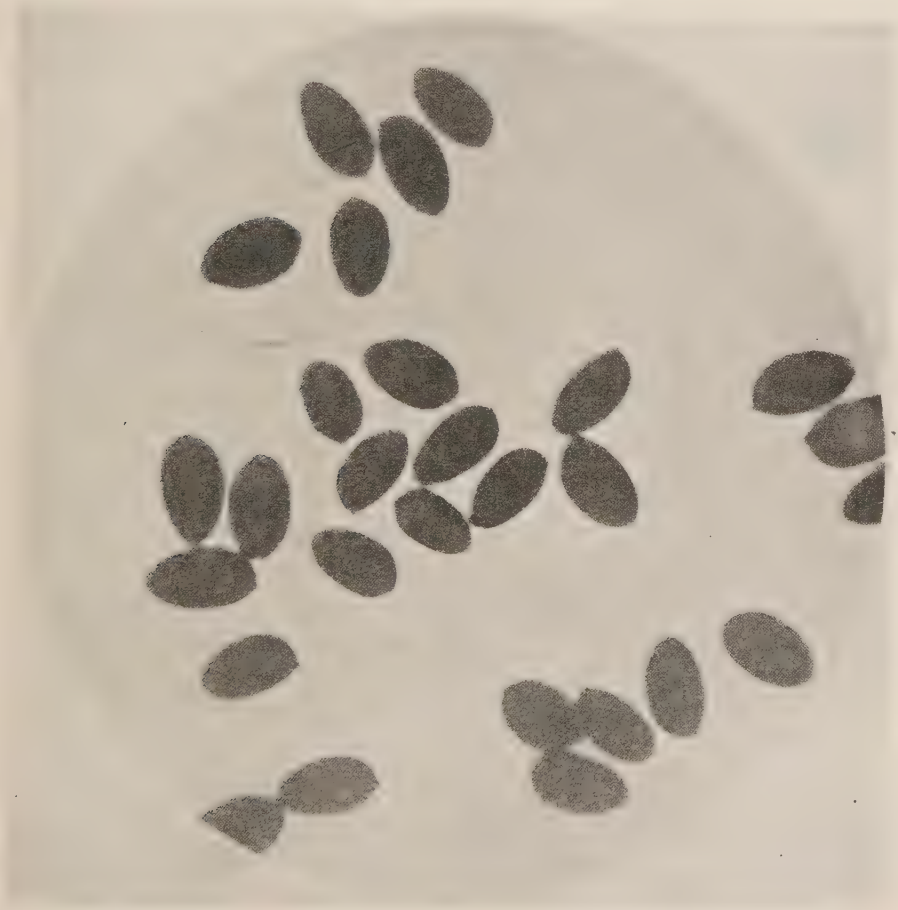


FIG. 92.—*Coprinus sterquilinus*. Photomicrograph of some living spores placed in water, showing their oval form and dense black wall. Here and there, at a spore's basal end can be seen the pointed spore-hilum, and at a spore's apex a light spot indicating the presence of an apical pore. A colourless wall-meniscus is faintly visible on the more sharply rounded side of each of the two uppermost central spores. Magnification, 650.

a whole basidium of a cultivated Mushroom (*Psalliota campestris*) with its two sterigmata and spores has a smaller volume. The rate of fall of the spores, since they are so large, must be relatively high. Spores of *Amanitopsis vaginata* which were spherical and

measured 11·65 μ in diameter were found to fall, immediately after their discharge, at the rate of 6·07 mm. per second.[1] Using this fact as a guide, I think it probable that the rate of fall of the undried spores of *Coprinus sterquilinus* varies from 6 to 8 mm. per second. But, after leaving the gills, the spores, if carried off by the wind in moderately dry air, would dry up within a few seconds and then would fall much more slowly. In any case, I think there can be no doubt that, on account of their great size, the spores of *Coprinus sterquilinus* are not carried far by light breezes. But this is of no serious disadvantage, as all that is required for successful dispersion is that the wind should carry the spores away from the fruit-body a distance sufficiently great to permit of their settling on grass along with which they may be swallowed by grazing horses. Since, under natural conditions, grass is usually present in the immediate vicinity of horse-dung masses upon which fruit-bodies have developed, the spores, although coming rapidly to earth after their liberation, are likely to lodge upon it. Wood-destroying fungi which live on trees usually have relatively small spores, and this makes it easier for the spores to settle in those high locations where invasion of the wood by their

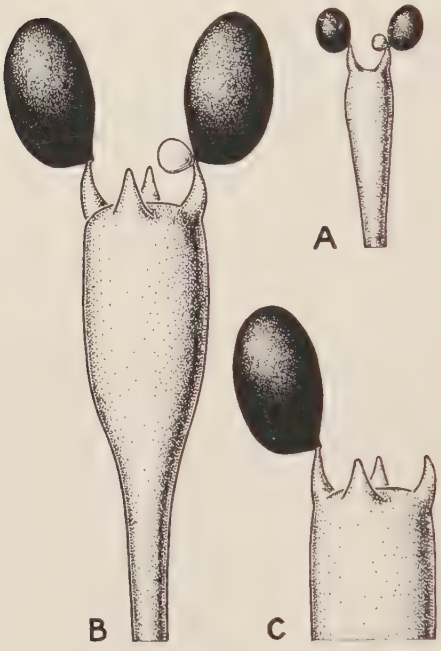


FIG. 93.—Comparative size of basidia. A, a basidium of *Psalliota campestris* (cultivated form); B and C, two basidia of *Coprinus sterquilinus*. The volume of the basidium and two spores of A is only about equal to the volume of a single spore of B or C. In A and B, each right-hand spore, in preparation for violent discharge, has just excreted a liquid drop. The size of the drops is proportional to the size of the spores. Magnification, 970.

[1] These *Researches*, vol. i, 1909, p. 173.

germ-tubes may take place. Were the spores of any wood-destroying fungus as large as those of *Coprinus sterquilinus*, they would be ill-suited as organs of dispersion for the species on account of their relatively rapid rate of fall and tendency to reach the ground quickly.

According to Fayod, in the majority of Agarics having coloured spores as well as in some having colourless spores (*Lepiota procera*), the wall of the spore consists of an outer colourless *exospore*, which previously had been overlooked, and an inner pigmented *endospore*.[1] Furthermore, Hansen [2] has demonstrated the presence of an outer colourless layer for the spore-wall of *Coprinus stercorarius*. He placed the spores in chlor-zinc iodine and observed that in a few minutes the outer colourless spore-wall became much swollen, whereas the inner pigmented wall remained unchanged; and I have repeated this experiment with the same result. The wall of the spore of *Coprinus sterquilinus*, when the spore is mounted in water, also appears to be double, *i.e.* to have a colourless exospore and a pigmented endospore; for, if a spore is exposed to view in profile in such a way that its hilum projects freely on its flatter dorsal side, one can readily perceive that, on its more rounded ventral side, there is a colourless layer of wall-substance covering the pigmented layer and somewhat resembling in form a median section taken through a concavo-convex lens (*vide* three of the five top spores in Fig. 92, p. 219).[3] However, when one attempts to trace this colourless layer all around the spore, a difficulty arises: one perceives a thin white layer extending along the flatter dorsal edge of the spore, but here there is the possibility that this layer is apparent and not real; for tiny air-bubbles the size of spores, seen in water, also appear to be enveloped by a thin sharp white layer, *i.e.* by a layer which, of course, is due to diffraction and therefore insubstantial. The outer wall of a spore of *Coprinus*

[1] V. Fayod, "Prodrome d'une histoire naturelle des Agaricinés," *Ann. de sc. nat.*, T. 9, 1889, p. 269.

[2] E. C. Hansen, "Biologische Untersuchungen über mist-bewohnende Pilze," *Bot. Zeit.*, 1897, pp. 111–132, Taf. II.

[3] The original photograph showed the colourless layer more clearly than does its reproduction in Fig. 92.

sterquilinus does not become swollen in chlor-zinc iodine, so that I have found it impossible to use that reagent for determining whether or not a colourless exospore completely envelopes an endospore.

As in all other Coprini and in chromosporous species of Agaricineae generally, in *Coprinus sterquilinus* there is an apical pore at the summit of the spore. At the mouth of this pore the wall is thinner than elsewhere and colourless; and, not improbably, it consists solely of exospore. The pore serves for the emission of a single germ-tube. Since there is but one pore, only one germ-tube is emitted. Where in the Hymenomycetes, *e.g. Polyporus squamosus*, the spore-wall is very thin, no pores are present, for none are required, and several germ-tubes may arise in diverse and variable positions at the spore's outer surface.[1] The spore of the chromosporous species of Agaricineae, which possesses thick and pigmented walls and one apical pore, and which emits a single germ-tube through the pore, is evidently much more specialised than the thin-walled colourless spore of most Leucosporae, which lacks a pore and emits germ-tubes varying both in number and place of origin. There seems every reason to believe that, in the course of evolution, the former has arisen from the latter and not *vice versa* as Massee once would have us believe.[2]

A few somewhat curious observations on the behaviour of unripe spores under certain artificial conditions may here be described. Some full-sized but colourless spores were removed from their sterigmata by rubbing a gill in a drop of water upon a glass slide. Some of these spores were then put in a hanging drop of water and others in a hanging drop of juice squeezed out from the gill. The spores in the water became strongly vacuolated and their walls remained colourless; but the spores in the juice from the gills continued their ripening processes, for in the course of seven hours their walls became dark brown. Subsequently some of these spores germinated.

[1] A. H. R. Buller, "The Biology of *Polyporus squamosus* Huds., a Timber-destroying Fungus," *Journal of Economic Biology*, vol. i, 1906, Plates VI and VII.

[2] G. Massee, "A Revision of the Genus Coprinus," *Ann. of Bot.*, vol. x, 1896, pp. 129–130.

A spore which has attained full size but is as yet colourless is connected by a channel through its hilum and the neck of the sterigma with the basidium-body from which it is receiving protoplasm and food materials. If now such an unripe spore is broken away from its sterigma under water, there is a hole in its wall at the hilum. Nevertheless, the spore does not collapse but remains living and apparently turgid; for one can strongly plasmolyse it with a solution of 10 per cent. potassium nitrate and then deplasmolyse it with water. This observation indicates that a spore, when shot away from its sterigma, does not require to have its hilum closed by a membrane. Closing walls at the hilum of a spore and at the end of the sterigma at the moment of spore-discharge have not as yet been definitely seen and possibly do not exist.

The number of spores on a fruit-body having a pileus 4 cm. high in the unexpanded condition, *i.e.* on a fruit-body of medium size, was estimated in the following manner. With the microscope it was found that on an area of the hymenium measuring 0·08 square mm. there were 58 basidia. Reckoning 4 spores to each basidium, it was then calculated that on 1 square mm. of hymenial surface there were 2,900 spores. After the area of the exterior surfaces of the gills had been measured, it was estimated that the hymenium had an area of 34,496 square mm. From these data it was calculated that the total number of spores on the whole pileus was 100,038,400 or, in round figures, 100,000,000. This number of spores, although so large when considered by itself, is relatively small; for a fruit-body of *Coprinus comatus*[1] was calculated to have produced 5,000,000,000 spores and a fruit-body of *Psalliota campestris*[2] 16,000,000,000. The small number of spores produced by *Coprinus sterquilinus* as compared with *C. comatus* is due to the fact that, in the former species, the fruit-body not only has a much smaller amount of hymenial surface but also a smaller number of basidia on each unit area of its hymenium. The relatively small number of basidia on each unit area of hymenium is correlated with the unusually large size of the basidia and their spores. A basidium of *C. sterquilinus*, since it is so much larger than a basidium of

[1] Vol. i, 1909, p. 83. [2] Vol. ii, 1922, pp. 403–404.

C. comatus, requires a proportionally increased amount of room for its development.

The Mode of Fall and the Adhesiveness of the Spores considered in relation with Herbivorous Animals.—The observations recorded in this Section were made by Mr. W. F. Hanna and the author working conjointly.

A spore of *Coprinus sterquilinus*, when shot away from its basidium, is fully turgid and has the appearance shown in Figs. 92 (p. 219), 93 (p. 220), and 95, A, B. As the spore falls through the air, the drop of water which was excreted at its hilum and which it carries upon its exterior soon evaporates and disappears (Fig. 95, C, D), and the spore, as a whole, takes up the position shown in Fig. 95, C, D, *i.e.* one in which the flatter side is uppermost and the more rounded side lowermost. This position may be considered one of stable equilibrium to which the spore must return if, after being disturbed therefrom, it is free to move.

If a spore, after being liberated, while it is still fully turgid, and before it has begun to shrink owing to loss of water, happens to fall on to a glass slide placed beneath the pileus or on to a blade of grass in a field, it usually comes to rest upon its more sharply rounded side (Fig. 95, E). Then, as the spore dries up (a process which may not take more than a few seconds), its upper flatter surface becomes depressed, and very rapidly the whole spore assumes a boat-shaped form (Fig. 95, F).[1]

Evidence which seems sufficient to prove that the spores of *Coprinus sterquilinus* do settle and dry up in the manner just described is readily obtained. One places a slide for two or three minutes close under a pileus which is shedding spores under a small bell-jar, obtains a thin spore-deposit, and then examines the deposit dry in face and side views under the microscope. (1) If, using a top light, one looks down on one of the dry spores from above (*cf.* Fig. 94), one perceives that the spore has an oval outline and is bilaterally symmetrical, that the spore-hilum is projecting upwards

[1] During the development of a spore, the flatter side of the spore is directed toward the basidium-axis and the more rounded side away from this axis (*cf.* Fig. 95, A, p. 227). When a spore dries up and becomes boat-shaped, it is always the flatter side of the spore which bulges inwards, and never the more rounded side; and this is what we should expect on physical grounds.

at one end of the spore in the line of the median spore-axis, and that there is a deep concave central depression in the spore surrounded by a broad and oval rim, so that one is reminded of a perforated indiarubber ball of which the upper side has been pushed downwards

FIG. 94.—*Coprinus sterquilinus*. A photograph of a thin dry spore-deposit collected upon a glass slide set a few inches beneath a pileus of a fruit-body growing on horse dung in the laboratory. The culture-dish, fruit-body, and slide were all covered by a bell-jar. The deposit is seen from above. All the spores are boat-shaped, with the hollows looking toward the observer; but, owing to the blackness of the spore-walls, the hollows cannot be seen in the photograph. This photograph shows the typical appearance of a spore-deposit taken from 1 inch to 6 feet below a pileus in still air. Photographed dry for W. F. Hanna and the author by A. E. Field. Magnification, 500.

from above. Owing to the blackness of the spore-wall, the central depression in each of the dried spores, in a photograph such as that reproduced in Fig. 94, shows either not at all or but faintly. The depression, as seen from above, has therefore been represented diagrammatically in Fig. 95, M. (2) If now one takes the slide bearing the spore-deposit and, instead of laying it flat, turns it through a right angle, fixes it in position on the stage with an iron

clamp, and then looks along the spore-bearing glass surface with the low power of the microscope, one sees that each spore appears to be flat or even slightly concave above and convex below, the hilum always being on the upper side of the spore and directed upwards (Fig. 95, F, L). A combination of the views described under (1) and (2) proves that each one of the dried spores has a boat-shaped form, its concave depression being directed upwards and its rounded keel downwards.

Hitherto, we have considered the case of a spore which has settled before it has undergone any contraction due to loss of water. However, in nature, owing to the air not being fully saturated with water vapour, it must usually happen that a liberated spore, immediately after being set free and long before coming to earth, undergoes partial or even almost complete desiccation. No doubt such a spore, whilst still being carried about by the wind, takes on a boat-shaped form (Fig. 95, G, H, I, J) exactly like that already described. A boat-shaped spore, suspended in the air, always falls with its hollow upwards and its rounded keel downwards (I, J), for in that position alone the whole spore is in a position of stable equilibrium. Experimental evidence supporting these assertions was obtained in the following manner. A box, six feet high and about half a square foot in transverse section, was set up in a room[1] which contained very dry air. To the top of the box, within, was pinned a pileus of *Coprinus sterquilinus* which was discharging spores and, on the bottom, was laid a glass slide. Then the box was closed, whereupon spores began to accumulate on the slide, so that a thin spore-deposit was soon formed. When the slide was examined under the microscope, it was found that the spore-deposit had exactly the same appearance as a spore-deposit (*cf.* Fig. 94) collected in damp air close beneath a pileus covered by a bell-jar, *i.e.* all the spores in it were boat-shaped and had their hollows directed upwards and their rounded keels downwards. Now, since, owing to their slow rate of fall in still air,[2] the spores must have taken upwards of five minutes to fall the six feet between the pileus and the slide upon which they came to rest, and since

[1] A warm laboratory, heated by steam radiators, in mid-winter, at Winnipeg.

[2] *Cf.* p. 220.

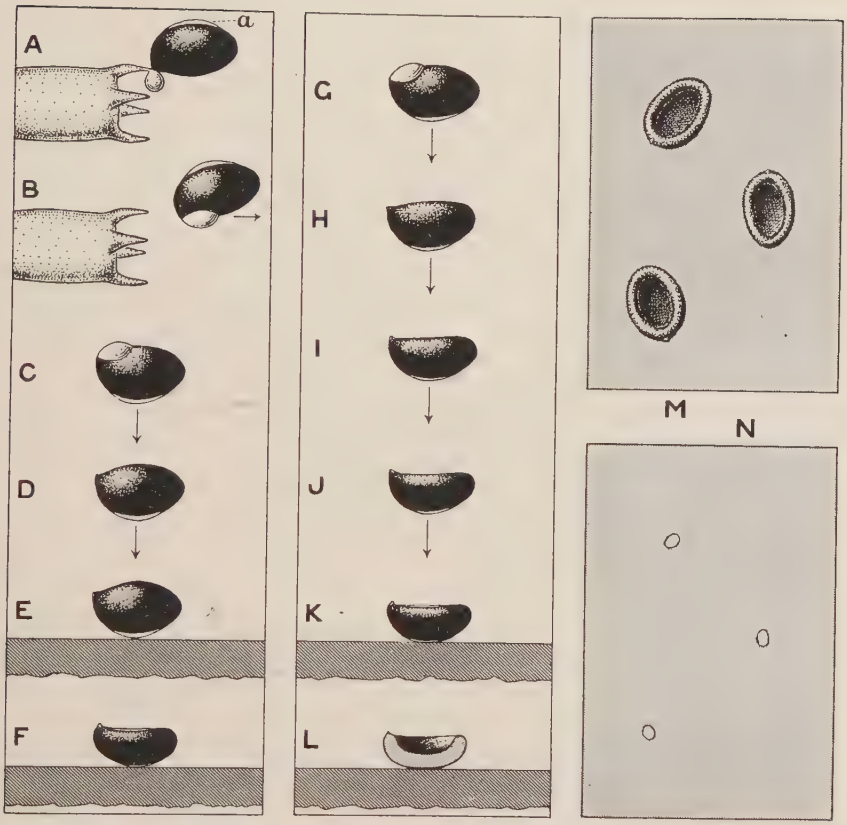


FIG. 95.—*Coprinus sterquilinus*. A diagram illustrating the manner in which a spore is discharged from its sterigma, falls through the air, settles on grass, etc., contracts on drying, and adheres to its substratum. A, the last spore of a basidium, about to be shot away from its sterigma. A drop of water is being excreted from the spore-hilum. On the more rounded side of the spore, away from the axis of the basidium, is a colourless wall-meniscus, *a*. B, the spore has just been shot away from its sterigma and, together with the adherent water-drop, is travelling horizontally in the direction shown by the arrow. C, the spore falling vertically downwards, as indicated by the arrow, in still, relatively moist air: the water-drop, the spore-hilum, and the flatter side of the spore are directed upwards, and the more rounded side of the spore, covered with its colourless wall-meniscus, is directed downwards. D, the spore still falling through the air: the water-drop has evaporated. E, the spore, still turgid and uncontracted, has just settled on a glass slide: the colourless wall-meniscus has come into contact with the surface of the glass. F, the spore after drying up: it is now boat-shaped, owing to the sinking inwards of its upper flatter side, and it is tightly attached to its substratum by its adhesive colourless wall-meniscus. G—K, a spore which becomes boat-shaped before settling. G, a spore, after being shot from its sterigma (as at A and B) has turned over (as at C) so that the water-drop, spore-hilum, and the flatter side of the spore are directed upwards and the more rounded side of the spores, covered with its colourless wall-meniscus, is directed downwards. H, I, J: the spore, still falling through the air, has lost its water-drop by evaporation and is drying and becoming boat-shaped. K, the boat-shaped spore has settled on a glass slide placed six feet below the pileus from which it was liberated, and it has now dried up: it is adhering to the surface of the glass by its colourless wall-meniscus. L, a vertical section through a dried boat-shaped spore such as those seen at F and K. M, three spores which settled upon, and became attached to, a dry glass slide, seen from above: they are now boat-shaped, the hollow of each boat facing the observer (*cf.* L). N, the same piece of glass slide after the three spores at M had been dislodged by pressing against them with the point of a dry needle: each spore has left behind a small piece of the colourless adhesive wall-meniscus attached to the surface of the glass. The wall-meniscus, except in N, has hitherto been observed only on spores immersed in water; but, since it exists, it has been shown attached to the spores in A—K, although these spores are represented as being in the air. Magnification, 351.

the air in the box was very dry, there can be no doubt that the spores must have lost most of their water and have become boat-shaped during their fall and, finally, must have settled on the glass slide hollow upwards and rounded keel downwards.

Another way of proving that a boat-shaped spore, whilst falling, has its hollow directed upwards and its rounded keel downwards is as follows. A model of a boat-shaped spore, 1–2 inches long, made of Japanese tissue paper, is liberated with its hollow downwards at a height of ten feet from the ground. One then observes that immediately after liberation the model turns over so that its hollow comes to look upwards and its rounded keel downwards. In the end the keel alone strikes the ground.

Fig. 96.—*Coprinus sterquilinus.* An abnormal spore-deposit collected upon a glass slide set a few inches beneath a pileus of a fruit-body growing on horse dung in the laboratory. Seen dry from above. The spores are in pairs and boat-shaped. In each pair the hollows of the spores face one another. For explanation see the text. Photographed dry for W. F. Hanna and the author by A. E. Field. Magnification, 500.

Normally, every thin dry spore-deposit of *Coprinus sterquilinus*, collected on a slide set one inch, six inches, or six feet beneath the pileus, seen with the microscope from above, has the appearance shown in Fig. 94 (p. 225): each boat-shaped spore has its hollow directed toward the observer and its keel directed away.[1] As a rare abnormality (only once observed), one may obtain a dry spore-deposit in which the boat-shaped spores occur not singly but in pairs, and rest not on their keels but on their sides, the hollows of each pair of spores facing one another (Fig. 96). This abnormality may be explained as follows. The water-drops excreted at the spore-hila of two or more spores situated on the same basidium became abnormally large, so that they touched one another and fused together. For a while the common drop enclosed between

[1] This is also true for spore-deposits collected within a few inches of the pileus of *Coprinus niveus, C. stercorarius, C. curtus, C. lagopus, Panaeolus campanulatus,* and *Stropharia semiglobata.*

the spores continued to grow (*vide infra*, Fig. 106, p. 250). Then one of the spores was violently propelled away from its sterigma and, in its flight, took with it part or the whole of the drop and another spore. The two spores, separated by the drop, after settling on the slide, dried up, and the spores were left on their sides in the position they first occupied when they fell on to the slide.

Now, as already mentioned in a previous Section,[1] the more rounded side of a spore of *Coprinus sterquilinus*, *i.e.* the side which is always directed downwards when the spore is falling through the air and which becomes the keel when the spore is drying and becoming boat-shaped, is covered by a colourless wall in the form of a concavo-convex meniscus (Fig. 95, *a* in A). Therefore, when a spore settles on a slide or on a blade of grass, *it is this colourless meniscus which comes into contact with the substratum* (Fig. 95, E, K). The meniscus is highly adhesive and, upon drying, sticks the spore very tightly to the substratum : for, when spores have settled and dried on a glass slide or a blade of grass, one cannot shake them off, however much violence may be used in the attempt and, as experiment showed, they cannot be dislodged by water in the form of continuous artificial rain, even when the rain is allowed to fall for twenty-four hours. As further evidence of the adhesiveness of the meniscus it may be mentioned that, if a spore has settled and dried on a glass slide and one dislodges it with a needle, a piece of the meniscus is often left behind on the surface of the glass, thus allowing one to perceive where the spore was attached before it was disturbed (Fig. 95, N).

The spores of *Coprinus sterquilinus* in a thin spore-deposit on a glass slide can be dislodged by a normal solution of sodium hydroxide but not by hot water, chloroform, alcohol, or concentrated nitric acid. Evidently the meniscus is not dissolved either by water or by many other chemical substances.

Coprinus sterquilinus, as we have seen,[2] is a coprophilous fungus which is normally found on horse dung. Now its occurrence on horse dung depends upon the fact that the spores settle on grass and are swallowed with the grass by horses : the spores pass uninjured down the alimentary canal of any horse which has swallowed

[1] *Vide* p. 221. [2] *Vide* p. 179.

them and emerge in the solid faeces where they germinate and produce a mycelium which, in the course of a few weeks, gives rise to new fruit-bodies and new crops of spores. This being so, it is of the greatest importance that the spores which have settled on the grass in pastures shall adhere very firmly to the grass and not be dislodged in the course of many weeks or even months by either violent winds or heavy rains. The adhesion of the spores, as a matter of fact, is secured (1) by the spores falling through the air with their more rounded side downwards, and (2) by their more rounded side, which comes into contact with the substratum, being provided with a very adhesive, colourless wall-meniscus which is insoluble in water. It thus appears that the presence of the colourless wall-meniscus on the exterior of a *C. sterquilinus* spore, the exact position of the meniscus, and the adhesive property with which the meniscus is endowed are all correlated with the part played in the life-history of the fungus by horses and other herbivorous animals.

The Discharge of the Spores and the Phenomenon of Autodigestion.—We have already seen that a wave of hymenial development passes from below upwards on each gill and that, as a consequence, the spores ripen in succession from below upwards. Whilst, owing to this ripening process, the gills are becoming black from below upwards, the pileus begins to expand (Fig. 97, B and C: also Fig. 75, p. 186); and, after the gills have become completely blackened from bottom to top, the pileus becomes campanulate (Fig. 97, D; also Fig. 76, p. 187).

When the pileus has become expanded to about the extent shown in Fig. 97, D, the process of spore-discharge begins. The photograph reproduced in Fig. 77 (p. 188) shows a fruit-body in which the discharge of spores had just begun. The first spores to be discharged are those which are situated in a narrow zone which extends along both sides of the extreme lower margin of each gill (*s* in Fig. 97, E). The zone of spore-discharge, after thus coming into existence, gradually moves upwards on each gill from the bottom to the top; and, in large fruit-bodies growing in the laboratory, it was observed to complete its ascent in from eight to twelve hours.

Just before spore-discharge begins, the marginal gill-flange (Figs. 78 and 79, pp. 189 and 191) beneath what is to become the zone of spore-discharge (*s* in Fig. 97, E) undergoes autodigestion. It breaks down, changing from a solid to a fluid condition ; and so it contracts and disappears. This autodigestive removal of the lower part of the gill-flange is very timely ; for, if the flange were to remain in existence until the process of spore-discharge began, by partially blocking the openings of the interlamellar spaces it would undoubtedly prevent many of the spores from escaping from the pileus. It is evident that the gill-flanges persist only so long as they are of service to the fruit-body by helping to maintain the interlamellar spaces during the development and ripening of the spores and that, after the pileus has begun to expand, when they become not merely functionless but also prospective mechanical obstacles to the fall of the spores, they are at once destroyed.

As soon as a narrow zone (about 0·1 mm. wide) along the bottom of each gill has become spore-free owing to spore-discharge from the basidia, the process of autodigestion which, as we have seen, has destroyed the gill-flange, involves this spore-free zone also. Each gill has two opposite spore-free zones upon it, one on each side ; and a band of the lower cells of the hymenium in both zones breaks down, becomes fluid, and is thus removed. The subhymenial and tramal cells between the two bands of hymenial cells suffer a like fate, so that the whole substance of the gill in a transverse direction becomes affected by the disintegrating process. All the gills are subjected to this local destruction at the same time.

The gradual change of the gills from the solid to the liquid condition which has been known to mycologists as *deliquescence* I have called *autodigestion*,[1] for there is every reason to suppose by analogy that the gill-tissues are destroyed by enzymes liberated from the cell-sap of the dying cells. This view has been supported by the observations of Weir [2] who found that the juice extracted from a pileus of a species of Coprinus contains an enzyme which is able to break down the walls of hyphae taken from the interior of the stipe.

[1] A. H. R. Buller, these *Researches*, vol. i, 1909, p. 200.

[2] J. R. Weir, "Untersuchungen über die Gattung Coprinus," *Flora*, Bd. 103, 1911, p. 271.

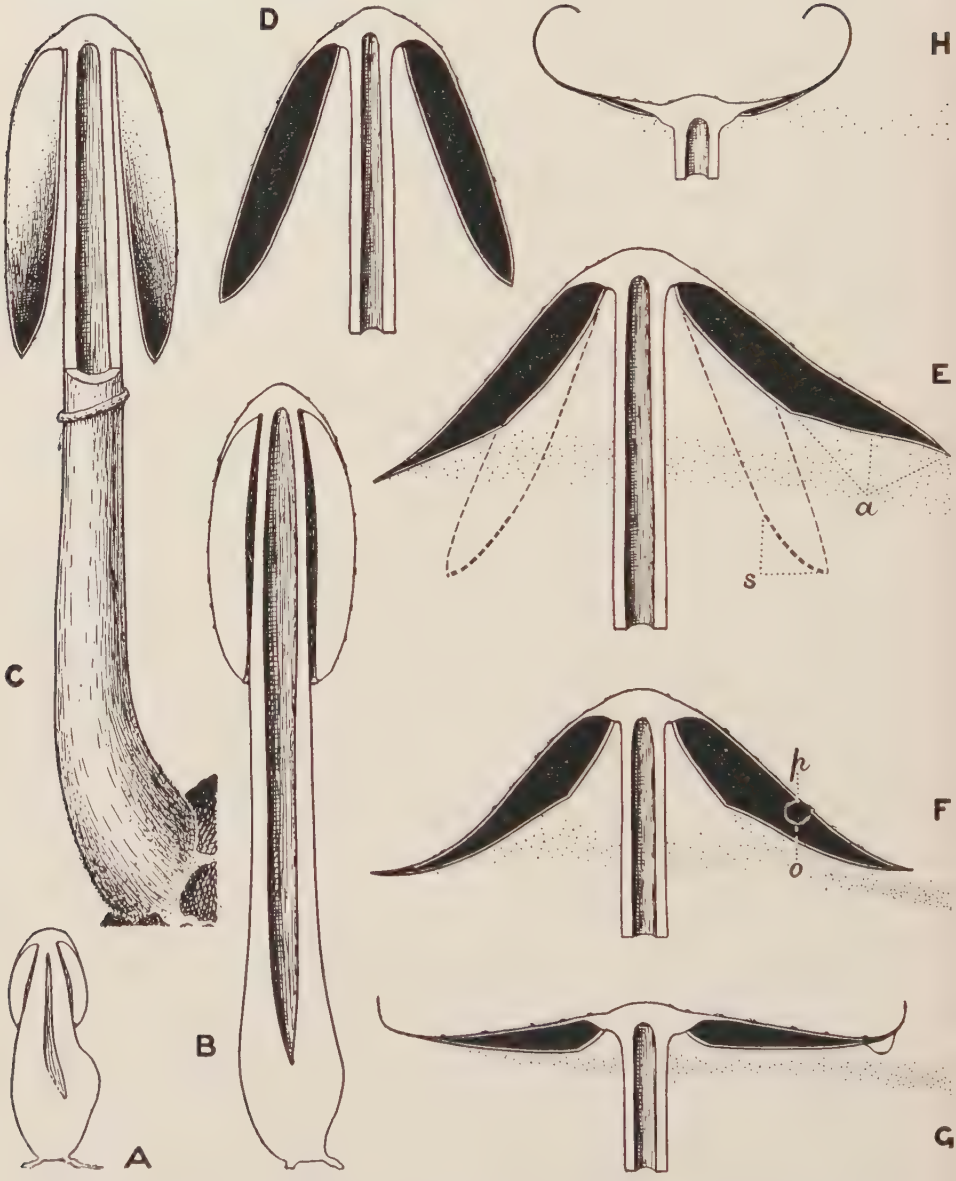


FIG. 97.—*Coprinus sterquilinus*. Stages in the development of the fruit-body as seen in vertical sections. A, a very young stage showing bulbous stipe and a pileus with white gills without spores in a gill-chamber. B, a stage two days later than A; the stipe is elongating by intercalary growth in the region of the pileus. The gills are white: the spores have been developed but their walls are still unpigmented. The annulus is being carried upwards on the base of the pileus. C, a fruit-body, a few hours older. The stipe, which is attached

The zone of autodigestion, which has come into existence in the manner explained above, gradually ascends each gill ; and thus all the gills are destroyed from below upwards. The zone of autodigestion follows hard after the zone of spore-discharge, but never invades it. It simply involves the lower part of the zone which has become spore-free.

After autodigestion has begun, five zones can be distinguished in succession from above downwards on the surface of each gill within half a millimetre of, and parallel to, its edge (Figs. 104 and 105, pp. 242 and 249) : (*a*) a zone of basidia with ripe spores which extends upwards toward the top of each gill and which therefore may be several mm. high, (*b*) a zone of spore-discharge composed of two sub-zones, (*c*) a zone of spore-free surface, (*d*) a zone of autodigestion, and (*e*), at the gill-edge, a dark adhesive liquid film containing the products of autodigestion and a certain number of spores which have failed to be properly discharged. These five zones keep their relative distances apart unaltered. They gradually move upwards, so that in large fruit-bodies, in the course of from eight to twelve hours, they involve the whole of each gill.

Fig. 97—*cont.*

below to a horse-dung ball, has attained almost its full height. The annulus has now been dropped by the pileus on to the stipe. In consequence of the ripening of the spores, the gills are turning black from below upwards. D, a section through an older pileus. In consequence of the spores having ripened, the gills have become black all over. Spore-discharge would begin in about half an hour. E, a section through a pileus about 1·5 hours after spore-discharge has begun. Spores are being shed and autodigestion is taking place along the gill-edge at *a*. The broken lines show the shape and extent of the gills at the moment when autodigestion began. *s*, the lower edge of the gill where spore-discharge and autodigestion first became active. About one-fifth of each gill has now been destroyed. F, a section through a still older fruit-body about 3·5 hours after the beginning of spore-discharge. The pileus has now become helmet-shaped through expansion. The gills have become reduced by autodigestion to about one-half their original size. A cloud of spores is being liberated at the gill-edges. The line *o*—*p* shows the direction in which a section was taken which is represented in Fig. 101, A (p. 237). G, a section through a still older fruit-body, about 5 hours after the beginning of spore-discharge. The pileus has become almost flattened at the top. The gills have now become reduced by autodigestion to about one-quarter of their original size. The exhausted parts of the gills have become recurved. On the right-hand side is seen a drop of brown fluid which has come into existence through autodigestion. The spores are shown diagrammatically escaping from the lower edges of the gills. H, section of a pileus in the last stage of its development, about 7·5 hours after the beginning of spore-discharge and about 8 hours after the stage shown at D. The gills have now become reduced by autodigestion to mere ridges. Spore-discharge is ceasing. The exhausted parts of the gills are turned upwards. The few spores still in course of being shed are shown diagrammatically, escaping from the lower edge of the gills. All natural size.

The shortening of the gills from below upwards in consequence of autodigestion is illustrated in Fig. 97, D to H (p. 232). In the pileus D, the gills are still complete. In the pileus E, about one-fifth of each gill has been destroyed. The zone of spore-discharge

FIG. 98.—*Coprinus sterquilinus*. A late stage in the expansion and autodigestion of the pileus. Spore-discharge is still very active (*cf.* Fig. 97, F and G, p. 232). The exhausted peripheral ends of the gills are becoming revolute. The blackness of the stipe is due to the presence of a black pigment in it and not to the lodgment of spores. From a pure culture on horse dung. Natural size.

FIG. 99.—*Coprinus sterquilinus*. A still later stage in the expansion of the pileus (*cf.* Fig. 97, G and H, p. 232). Spore-discharge is nearly completed. The autodigestion of the gills took place under damp conditions (under a bell-jar). Hence its liquid products have accumulated as drops at the periphery of the pileus; but they are out of the way of the stream of spores still falling. The rim of the pileus is still more revolute. The gills are now almost completely destroyed but are still discharging spores from their edges. Natural size.

is just above the gill-edge region indicated by the letter *a*. Broken lines show the shape and position of the gills just after spore-discharge began. The first spores to be liberated were set free just above the gill-edge at *s*. The pileus has evidently expanded considerably since spore-discharge began. In the pileus F, about

one-half of each gill has disappeared, in G about three-quarters, and in H nearly the whole. In the drawings E, F, G, and H, the spores have been represented diagrammatically as falling from the gill-edges and being carried away by a gentle current of air. In the drawings, B, C, D, E, F, G, and H, one may follow the gradual expansion of the pileus just before and during the discharge of the spores : one perceives that the pileus, which is at first barrel-shaped (B), becomes campanulate (C, D), then helmet-shaped (E, F), then flattened (G), and finally revolute (H). The revolution of the rim of the pileus serves to remove the exhausted and therefore now functionless peripheral parts of the gills out of the way of the stream of spores which is being carried off by the wind. The spore-stream of the revolute pileus shown at H is coming from the edges of the much reduced but still active parts of the gills which stand in a ring around the top of the stipe immediately beneath the disc.

The relation of certain photographs of pilei to the vertical sections of Fig. 97 may now be considered. The photograph reproduced in Fig. 77 (p. 188) shows a pileus which had just begun to shed its spores and which therefore is very slightly in advance of the pileus shown at D in Fig. 97 (p. 232) which is just about to begin to shed its spores. The photograph reproduced in Fig. 98 (p. 234) shows a pileus at a stage corresponding to the stage F in Fig. 97, *i.e.* a middle stage in spore-discharge and autodigestion ; whilst the photograph reproduced in Fig. 99 (p. 234) shows a pileus at a stage corresponding to the stage H in Fig. 97, *i.e.* a stage in which the spore-discharge period is almost ended and in which the process of autodigestion has almost destroyed the gills. The photograph reproduced in Fig. 100 shows, two-thirds the natural size, the whole of the fruit-body from which the pileus of Fig. 99 was taken. The exposed upper part of the stipe and the ragged, revolute, dripping, almost exhausted pileus of Fig. 100 contrast strikingly with the half-concealed upper part of the stipe and the intact campanulate pileus of the younger fruit-body shown in Fig. 77 (p. 188). In the latter fruit-body, as already pointed out, the liberation of the spores and the gradual destruction of the gills by autodigestion had only just begun.

Fig. 100.—*Coprinus sterquilinus*. Fruit-body in a very late stage of development, growing on sterilised horse dung in a glass dish (same fruit-body as that shown in Fig. 99). The pileus has become torn into rays, some of which are weighed down by dark drops—the liquid products of the autodigestion of the gills. The stipe is abnormally twisted. The blackness of its upper portion is due to a black pigment and not to the deposition of spores. However there are some spores on the upper side of the stipe just above the dung. Reduced to $\frac{2}{3}$ natural size.

In Fig. 101 at A is shown a vertical section through three gills taken in the direction *o*—*p* in Fig. 97, F (p. 232); and in Fig. 101 at B is shown a small piece of a gill in surface view (*cf.* the surface view of the right-hand gill in Fig. 97, F in the region crossed by the line *o*—*p*). In both A and B the magnification is only 13 diameters, and yet the spores on the hymenium are clearly distinguishable owing to their extremely large size. The arrows show the directions of movement of the spores as these are shot outwards from the zone of spore-discharge into the interlamellar spaces in still air. In the drawing A, one may observe: the subparallel-sidedness of the gills, the splitting of the gills from above downwards, the broad interlamellar spaces which reference to the scale shows to be about 1 mm. wide, and that the

spores are shot only about 0·2 mm. into the interlamellar spaces before they begin to fall vertically. In the drawing B, the spores have been introduced in their correct size, distribution, and number, and a glance at it will help one to realise the enormous number of spores which may be borne on even a single gill. Below the ripe spores, which darken the surface of the hymenium, is

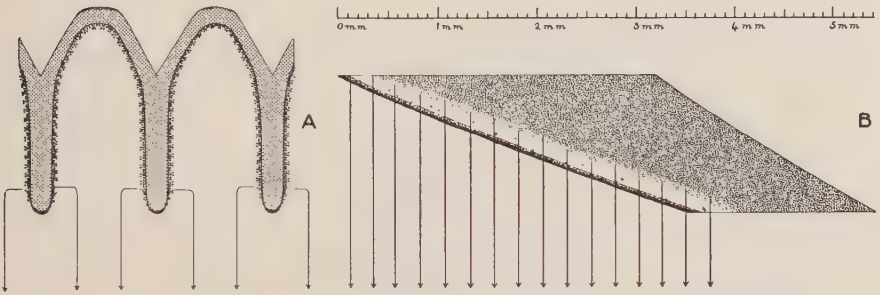


Fig. 101.—*Coprinus sterquilinus*. A, vertical transverse section through three gills of an expanded pileus which is shedding spores. The section has been taken in the direction indicated by the line *o*—*p* in Fig. 97, F (p. 232). The gills are split for a certain distance from above downwards through their median planes. The trajectories of a few spores being shot away in the zones of spore-discharge have been indicated. B, a surface view of a piece of a gill of an expanded pileus which is shedding spores. It corresponds to a lateral view of one of the gills shown in A or to a surface view of part of the right-hand gill shown in Fig. 97, F, in the region *o*—*p* (p. 232). The spores are being shed from the zone of spore-discharge just above the gill-edge. At the extreme gill-edge, the black line indicates the wasted spores (*cf.* Fig. 104, p. 242, zone *e*). The light zone above includes the spore-free zone and the zone of autodigestion. The upper ends of the arrows show the position of the zone of spore-discharge. The arrows show the direction of fall of a few spores in still air. Above the arrows the gill is covered with basidia which have not yet discharged their spores. Magnification, 13.

seen the zone of spore-discharge from which spores are being shot away. Below this zone is a white band representing the spore-free zone and the zone of autodigestion; while, at the extreme gill-edge, is the zone of the products of autodigestion—a liquid film containing many spores which failed to be properly discharged.

The spore-deposit of *Coprinus sterquilinus* illustrated in Fig. 102 was made as follows. A pileus which had attained to the stage of development shown at F in Fig. 97 (p. 232) and in Fig. 98 (p. 234) was detached from its stipe, set on a piece of white paper, covered with a small inverted crystallising dish, and removed at the end of

about fifteen minutes. Owing to the presence of the dish, the spores were discharged from the gills in still air. The peripheral smudges correspond merely to places where the autodigesting edges of the gills rested on the paper. Each gill shot away spores from its two opposite spore-discharge zones close to the gill-edge. Hence the spore-deposit from each gill is shown in the figure as a pair of radially running, parallel, even, black lines, each about 0·3 mm. thick. The white line between the pair of black ones corresponds to the gill's extreme edge, which was undergoing autodigestion and not discharging spores. The remaining white spaces, which widen as they proceed centrifugally and which vary from about 1 to 2·5 mm. in width, correspond to higher parts of the gills arched over the interlamellar spaces (*cf.* Fig. 101, A, p. 237), from which no spores were as yet being discharged.

FIG. 102.—*Coprinus sterquilinus.* A spore-deposit made by a large expanded pileus (*cf.* Fig. 97, F, p. 232) in 15 minutes. The pileus was removed from its stipe, set on white paper, and covered with a small inverted crystallising dish. The peripheral smudges are due to contact of the autodigesting gill-edges with the paper. Each gill shot away spores from its two opposite spore-discharge zones just above its free edge; and the spores, after travelling horizontally not more than 0·2 mm., fell vertically downwards. Hence each gill is represented by a thin white line bounded by two thin black ones. Above the broad white radial bands, the hymenium had not yet begun to shed spores. Natural size.

The Aequi-hymeniiferae or Non-Coprinus fungi shed spores simultaneously from all parts (every square mm.) of their gills and not simply from very narrow zones of spore-discharge at their gill-edges. Hence their spore-deposits show only two features: narrow, radial, spore-less lines corresponding to the gill-edges and

broader, radial, spore-filled areas (uniformly coloured if the spores are coloured) corresponding to the interlamellar spaces in their entirety. The difference between the appearance of an inaequi-hymeniiferous spore-deposit and an aequi-hymeniiferous one, may be at once realised by comparing Fig. 102, which shows a spore-deposit for *Coprinus sterquilinus*, with Fig. 141 in Volume II (p. 402), which shows a spore-deposit for *Psalliota campestris*.

The five zones which can be distinguished near any gill-edge from which spores are being liberated are illustrated with a magnification of 300 diameters in Figs. 104 and 105 (pp. 242 and 249), and they will now be described in detail.

(*a*) *The zone of the basidia with ripe spores.* This zone has been fully described already in the Section dealing with *The Structure and Development of the Hymenium.* As we have seen, it is composed of long and short basidia and of paraphyses, so arranged as to form a beautifully efficient hymenial mosaic-work. All these elements are clearly shown in the zone *a* in Figs. 104 and 105. The spores of the long and the short basidia are equal in size and quite black, but in Fig. 104 the spores of the long basidia have been shaded more deeply than those of the short basidia for the purpose of giving a correct perspective : the former are at a higher plane than the latter and therefore stand nearer to the reader. The long basidia are almost as numerous as the short ones, and the spores of the former often stand in part vertically over the spores of the latter.

(*b*) *The zone of spore-discharge.* We have seen that, when the mature hymenium is about to shed spores, the basidia are crowded together in such a way that the spores of the long basidia often stand in part vertically over some of the spores of the short basidia. If, in the zone of spore-discharge, the spores of the short basidia were to be shot off before, or at the same time as, the spores of the neighbouring long basidia, it is obvious that the former would often collide with the latter and on this account be prevented from freely escaping from the fruit-body. Such collisions, however, are prevented owing to the fact that the long basidia shoot away their spores just before the short ones which are immediately below them. The zone of spore-discharge is always composed of two sub-zones : (1) an upper zone, b_1 in Figs. 104 and 105 (pp. 242 and 249), in which

the long basidia alone are discharging their spores, and (2) a lower zone, b_2, in which the short basidia alone are shedding spores. The two zones together are about 0·12 mm. wide.

The means employed for observing the order of spore-discharge just described was as follows. An ordinary microscope was fitted

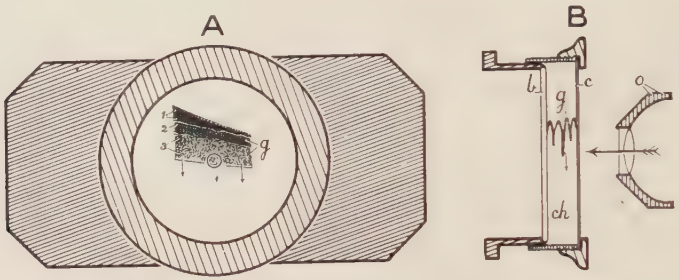


FIG. 103.—Apparatus used for observing spore-discharge from long and short basidia of *Coprinus sterquilinus*. A, a compressor cell, set vertically upright on the stage of a microscope tilted to an angle of 90°. The chamber contains a piece of pileus with the gills, *g*, looking downwards as under natural conditions. Of the gills nos. 1 and 2 have been cut short, while no. 3 is complete to its lower edge where it is discharging spores and undergoing autodigestion from below upwards. The arrows indicate the trajectories of some of the falling spores, and the central circle the field of view seen through the low-power objective of the microscope. B, a vertical transverse section through the middle of A: *ch*, the chamber of the compressor cell bounded in front by the cover-glass *c* and behind by the glass base *b*, both held in brass frames; *g*, a piece of the pileus held by pressure between *b* and *c*, having all the gills cut short except the central one which was discharging spores at its free edge; the tiny arrow indicates the trajectory of a single spore shot out from the zone of spore-discharge; *o*, the end of the low-power objective of the microscope. The horizontal arrow indicates the direction of vision. Natural size.

with a low-power objective. Its body was then turned about the inclination-joint at the top of the pillar through a right angle, with the result that the tube came to have its axis set in a horizontal direction and the stage to lie in a vertical plane (Fig. 103, B, *o*). Half a gill which was actively discharging spores was then removed from a fruit-body growing in the laboratory and suspended by its upper edge in a compressor cell clamped to the stage of the microscope (Fig. 103, A and B). Thus the piece of gill had an orientation in space similar to that which it had when forming part of the fruit-body from which it was taken; and, at the same time, it could

be readily observed through the horizontal tube of the microscope. The magnification employed was 100 diameters.

The lower edge of the piece of gill, on being brought into focus, could be seen very clearly, owing to its transparency, the large size of the hymenial elements, and the contrast made by the black spores against their white background. The zone of spore-discharge presented the appearance shown in Fig. 104 between the brackets b_1—b_1 and b_2—b_2; and it was actively liberating its spores, for spores could be seen raining down past the gill-edge. It was observed that the four spores of each active basidium disappeared from their sterigmata one after another in succession in the course of about 1–5 minutes, as in other Hymenomycetes.

On examining the zone of spore-discharge carefully, I discovered that it was necessary to distinguish between (1) a higher sub-zone (b_1—b_1 in Fig. 104, p. 242) in which the long basidia only were discharging their spores and (2) a lower sub-zone (b_2—b_2) in which the short basidia only were discharging their spores. In the latter sub-zone, the long basidia no longer bore spores. It was observed that, as the whole zone of spore-discharge gradually travelled up the gill, the two sub-zones kept the same relative distance apart. If in Fig. 104, which embodies the results of the observations here recorded, the sub-zone b_1—b_1 be carefully scrutinised, it will be noticed that the long basidia are shedding their spores in succession from below upwards; for some of them have already lost one, two, or even three of their spores, while the water-drops (w) on the hila of some of the spores indicate by their presence that the spores to which they are attached are within a few seconds of being discharged. In the sub-zone b_2—b_2 we have a repetition for the short basidia of the phenomena of spore-discharge which have just been described for the long basidia: the short basidia are shedding their spores in succession from below upwards; and water-drops are appearing at the hila of spores which are about to be discharged.

The direct observations on a living gill which have just been described prove beyond doubt that, *in the general zone of spore-discharge, the long basidia shoot away their spores just before the short basidia below them.* That the long basidia should thus take precedence over the short at the climax of their existence is a most

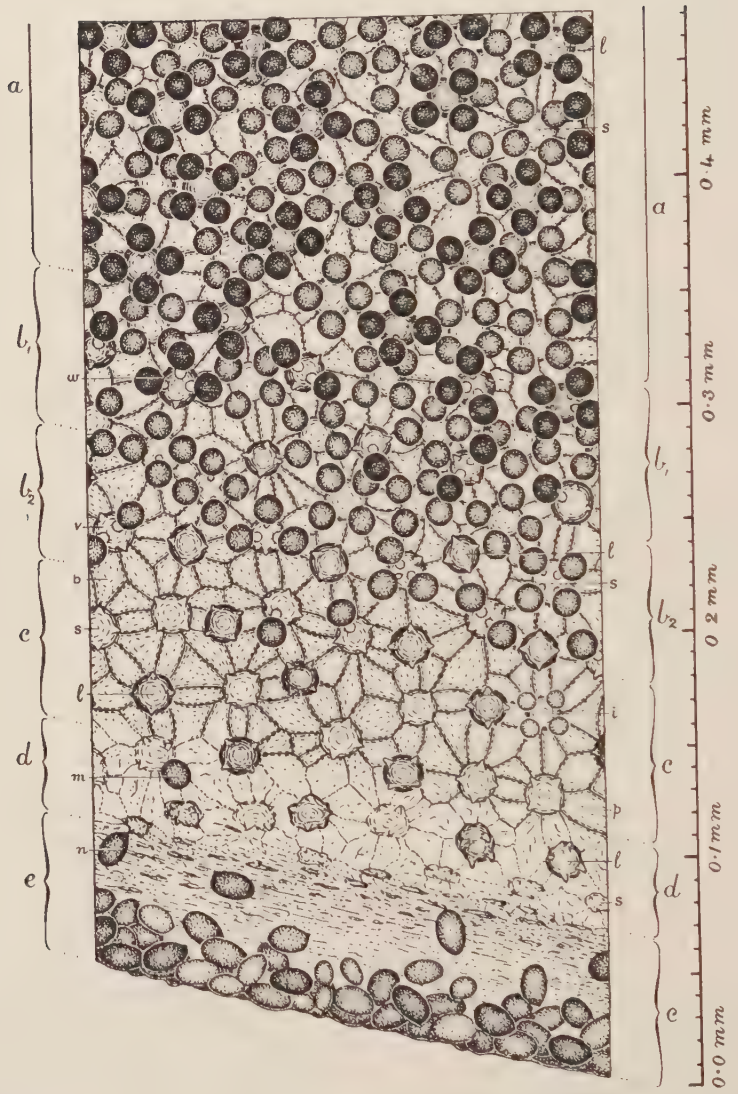


FIG. 104.—*Coprinus sterquilinus.* This drawing corresponds to the section given in Fig. 105. It shows the appearance of part of a gill-surface, including the gill-edge, after spore-discharge and autodigestion have begun (surface views anywhere just above the gill-edge region indicated by *a* in Fig. 97, E, p. 232, would have this appearance). There are five zones running parallel to the oblique gill-edge: (1) *a*—*a*, zone of basidia with ripe spores. The basidia are dimorphic, long and short. The spores of the long basidia, which are more deeply shaded, often stand in part above spores of the short basidia. The paraphyses are permanently sterile elements which separate adjacent basidia and prevent mutual jostling. (2) b_1—b_1 and b_2—b_2, together, form the zone of spore-discharge where the basidia are discharging their spores into an interlamellar

beautiful refinement in the organisation of the hymenium; for, obviously, it is correlated with the crowding together of the dimorphic basidia and *serves to prevent the spores of the short basidia colliding with, and adhering to, the spores of the long basidia during their discharge.* Here again, we have clear evidence of the high efficiency with which the fruit-body of a Coprinus performs it one great function of producing and liberating spores.

Fig. 105 (p. 249) represents a vertical transverse section through the lower part of a gill corresponding to the surface view of Fig. 104 and allows us to study the zone of spore-discharge from a new angle. The spores about to be discharged are represented with arrows in front of them, the arrows indicating the directions in which the spores will travel away from the hymenium. Here, as we have seen in the surface view, the sub-zone of spore-discharge for the long basidia, b_1, lies at a higher level than the sub-zone for the short basidia, b_2. Thus, on the left-hand side of the figure, the long

FIG. 104—*cont.*

space. There are two sub-zones: b_1—b_1, the zone of spore-discharge for the long basidia; and b_2—b_2, the zone of spore-discharge for the short basidia. In b_1—b_1, the four spores of each long basidium are shot off their sterigmata in succession, so that in this zone some of the long basidia have three spores left upon them, some two, and some one. A few seconds before a spore is shot away, a small drop of water is excreted at the top of the sterigma. This drop is represented as projecting toward the axis of the basidium beneath many of the spores, *e.g.* in the basidium *w*. In the sub-zone b_1—b_1, none of the short basidia have shed their spores. In the sub-zone of spore-discharge for the short basidia, b_2—b_2, the long basidia, *l*, no longer bear any spores; but they can be distinguished by their heavier shading and by their vacant sterigmata. The short basidia only are discharging spores. Some of them have three spores on, some two, and some one. Just before the discharge of each spore, a drop of water similar to that already described in connection with the sub-zone b_1—b_1, is excreted at the top of each sterigma, *e.g.* as at *v*. (3) *c*—*c*, the zone of basidia which have shed all their spores. Here both long and short basidia, normally, have shed all their spores. One basidium, *i*, still has four spores upon it, but these are immature and are destined eventually to join the wasted spores at the extreme gill-edge. *l*, a long basidium; *s*, a short basidium; *p*, a paraphysis. (4) *d*—*d*, the zone of autodigestion. The basidia and paraphyses are becoming indistinct. The walls are breaking down into fine granules. The spores *m* and *n* failed to be discharged at the proper time and are destined to join the other wasted spores at the extreme gill-edge. *l*, a long basidium; *s*, a short basidium. (5) *e*—*e*, the liquid film at the gill-edge containing the products of autodigestion and the spores which, for one reason or other, failed to be discharged in a normal manner. At the top of this zone, the collapsed basidia can still be dimly traced by rows of granules. Deeper down in the zone, the products of autodigestion are more homogeneous. At the gill-edge are a large number of undischarged or wasted spores. Some have a normal appearance; while others are above or below normal size, or are colourless, or not completely pigmented. They form but a small percentage of the whole number of spores. As the zone rises, it will add to itself the spores *m* and *n* and those at *i*. The scale on the right-hand side of the Figure shows the dimensions of every part.

basidium w in the sub-zone b_1 will discharge all its spores before the short basidium r immediately below it shoots away its first spore. On the right-hand side of the figure, the numbers 1 to 7 contained within the brackets b and a and opposite the basidia indicate the order in which the basidia will discharge their spores. A study of this order will show the reader how systematically the spores of the long basidia are removed before the time comes for the discharge of the spores of the immediately neighbouring short basidia. Water-drops are represented as being excreted from the hila of certain spores of the basidia v and w on the left-hand side of the figure and of the basidia No. 2 and No. 3 on the right-hand side. The scale shows that the zone of spore-discharge is only 0·2 to 0·3 mm. from the extreme edge of the gill.

When observing the edge of the hanging gill in the compressor cell with the horizontal microscope, I selected certain groups of basidia for especial study. In one such group, situated just above the approaching zone of spore-discharge (*cf.* Fig. 104, zone a, p. 242), a central long basidium was projecting beyond, and surrounded by, four short basidia, two above and two below. The arrangement of these five basidia is shown diagrammatically in the first column of the subjacent Table. For convenience, the central long basidium will be referred to as A, the two lower short basidia as B and C, and the two upper short basidia as D and E respectively. The five basidia soon became involved in the upward-moving zone of spore-discharge, and I succeeded in observing the successful discharge of all the twenty spores belonging to them. The moment of discharge of each spore was carefully noted. I called out as each spore was shot away, and my assistant, Mr. S. G. Churchward, recorded the times in seconds by his watch. If the time of discharge of the first spore on each basidium be taken as the zero of a time-scale and the times of discharge of the other three spores be reckoned from it, then the Table on p. 245 shows the times of discharge for all the four spores of each of the five basidia.

From the data there given it will be seen that each of the five basidia shot away its four spores in less than a minute and a half after the first spore had been shot away, and that the mean time occupied in discharging the four spores was just over one minute.

The Rate of Discharge of the Four Spores for each of Five Basidia of Coprinus sterquilinus

Relative Positions upon the Gill of the Five Basidia.	Basidium.	Time in Seconds at which the Four Spores were Discharged.			
		First Spore.	Second Spore.	Third Spore.	Fourth Spore.
D E	A, long, in centre . .	0	25	45	69
	B, short, left lower . .	0	25	32	42
A	C, short, right lower . .	0	10	17	80
	D, short, left upper . .	0	1	55	77
B C	E, short, right upper . .	0	5	54	68
	Average for the five basidia .	0	13·2	40·6	67·2

The order in which the five basidia began to shed their spores is indicated by their alphabetical arrangement. The long basidium A first discharged all its four spores. After the last spore had gone there was a pause for 45 seconds. Then basidium B, one of the lower short basidia, shot away its first spore. After 32 more seconds had passed the other lower short basidium, C, shot away its first spore. Thus the two lower short basidia, B and C, waited 45 and 77 seconds respectively after the long basidium above them had shed its last spore before shooting away their first spores. Owing to this delay, all the spores of these two short basidia were shot away without colliding with any of the spores of the long basidium. Whilst the two lower short basidia were shedding their spores, another long basidium above the two upper short basidia, which we will call F, was discharging its spores. Thus by the time that the two lower short basidia had completed the discharge of their spores, spores of the long basidium F were no longer overlapping the spores of the two upper short basidia. The two upper short basidia D and E shot away their first spores 10 and 85 seconds respectively after the last spore of the two lower short basidia had been discharged. If the moment of discharge of the first spore of the central long basidium A be taken as the zero of a time-scale, then the times of discharge of all the twenty spores of the five basidia, A to E, are as indicated in the following Table.

Order of the Discharge of Spores of One Long Basidium and of Four Surrounding Short Basidia in Coprinus sterquilinus

Basidium.	Time in Seconds at which the Four Spores were Discharged.				Mean Time of Discharge for all the Four Spores of each Basidium.
	First Spore.	Second Spore.	Third Spore.	Fourth Spore.	
A, long, in centre .	0	25	45	69	34·75 seconds
B, short, left lower .	114	139	146	156	2 mins. 18·75 secs.
C, short, right lower .	146	156	163	226	2 mins. 52·75 secs.
D, short, left upper .	236	236	291	313	4 mins. 29 secs.
E, short, right upper .	311	316	365	379	5 mins. 42·75 secs.

By comparing the time of discharge of the last spore of the basidium A with the times of discharge of the first spores of the basidia B and C, D and E, we may draw the conclusion that a long basidium completes the discharge of its spores about one minute before the short basidia immediately below it begin to shed their spores and about three minutes before the short basidia immediately above it begin to shed their spores. Generalising further, we may say that, in the zone of spore-discharge, the discharge of the spores of a long basidium precedes the beginning of the discharge of the short basidia by which it is surrounded by about one to three minutes. The long basidia therefore shoot away their spores only just in time to clear them out of the way of the spores of the short basidia when these come to be discharged.

In the Section upon *The Structure and Development of the Hymenium*, it was shown that the long basidia represent a first generation of basidia, and the short a second generation, and that the long basidia commence to develop their spores about two hours before the immediately neighbouring short basidia. This lead, however, becomes gradually reduced during subsequent development with the result that, as we have just seen, the long basidia precede the short basidia in the time of discharge of the spores by an interval of less than five minutes.

In order to study the discharge of the spores with a higher magnification than was possible when the compressor-cell method was employed, I frequently proceeded as follows. A gill was

removed from a living fruit-body and placed flat on a glass slide without any mounting fluid. A cover-glass was then laid over it. As the cover-glass touched the surface of the gill only in a few places, a large part of the zone of spore-discharge was left undisturbed. Under these conditions, I was able to examine this zone with the high power of the microscope ; and, with a magnification of 440, I succeeded in observing the discharge of a considerable number of spores. Sometimes, however, the under side of the cover-glass became fogged owing to the condensation of water-vapour upon it ; and then the preparation was spoiled. In successful preparations, it was found that a drop of fluid was always excreted from the hilum of each spore just before spore-discharge was consummated ; and a number of such drops have been introduced in the zones of spore-discharge represented in Figs. 104 and 105 (pp. 242 and 249). A drop, after its first formation, continues to grow for from 15 to 30 seconds, and at the end of this time it has attained its maximum diameter which is equal to from one-third to one-half the diameter of the spore. As soon as the drop has become of full size, the spore is discharged. As the spore disappears, the drop disappears too, so that it can no longer be seen at the end of the sterigma. Doubtless it is carried away by the spore in the manner which has been described for other species.[1] In a number of other Hymenomycetes with smaller spores, the excreted drop grows only for from 5 to 10 seconds, and then discharge takes place. *Coprinus sterquilinus* possesses unusually large spores and correspondingly large drops. Possibly it is on this account that the growth of the drops takes the unusually long time of 15 to 30 seconds.

It was sometimes observed in my preparations that, when a fruit-body had been grown under conditions in which the atmosphere around it was saturated with water-vapour, some or most of the spores in the zone of spore-discharge failed to be shot away. Drops were seen to appear in the normal manner (Fig. 106, A), and they rapidly grew to the normal maximum size (B) ; but then, instead of the spores being discharged, the drops continued to grow in volume. They soon became so big that they stepped up from the

[1] These *Researches*, vol. ii, 1922, pp. 14–17.

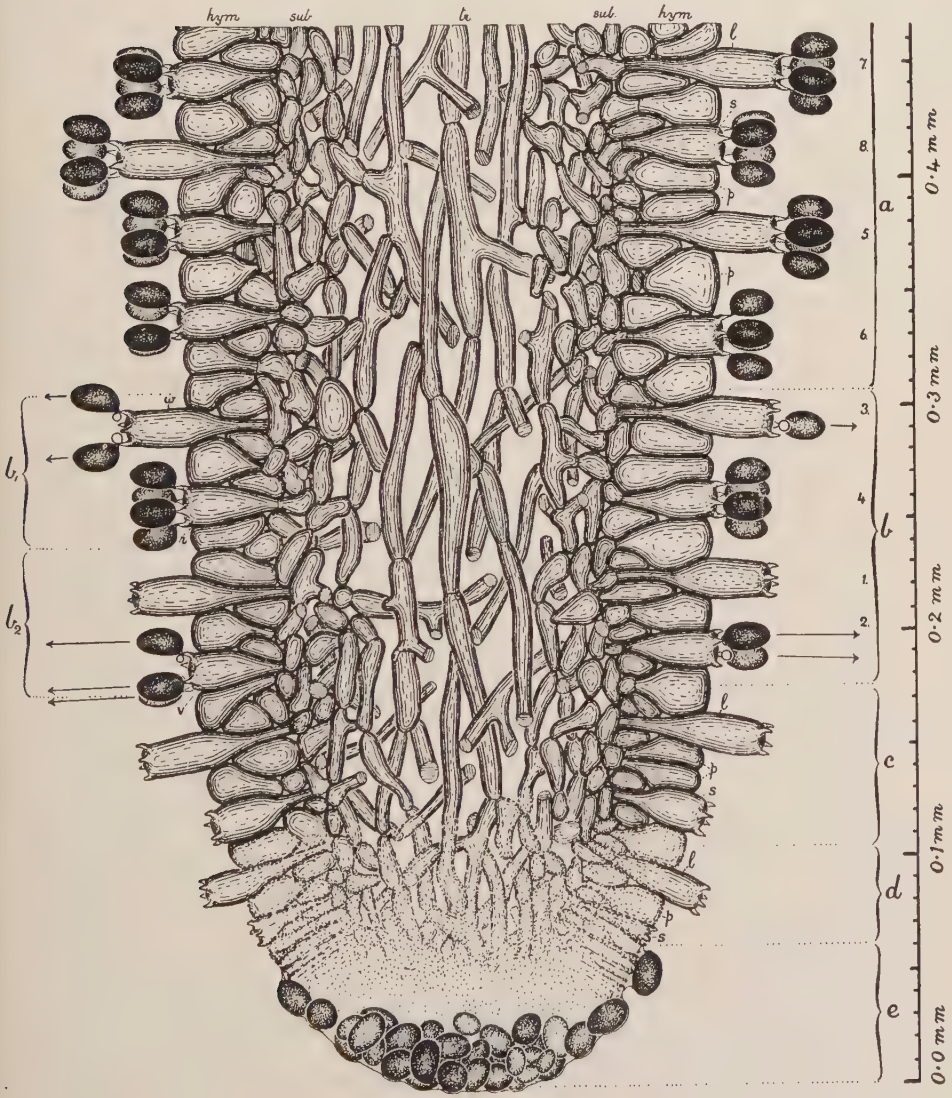
hila of the spores on to the sides of the spores (C). They then continued to grow until they touched one another (D). On touching, the four drops suddenly fused into one large drop which, owing to surface tension, dragged all the four spores together (E). After this, excretion usually ceased, and then the drop slowly diminished in size owing to evaporation. Eventually the four spores collapsed together and became stuck to the general surface of the hymenium

FIG. 105.—*Coprinus sterquilinus*. This drawing corresponds to the surface view of Fig. 104 and is drawn to the same scale, so as to allow of minute comparison. It shows the appearance of a section taken through the lower part of a gill including the gill-edge, after spore-discharge and autodigestion have begun. *Hym*, the hymenium; *sub*, the subhymenium; *tr*, trama. As in Fig. 104, five zones are represented: (1) *a*, the zone of basidia with ripe spores. Here the dimorphism of the basidia is very obvious. On the right-hand side of the Figure, two long basidia are shown alternating with two short. Although one of the spores of the long basidium, *l*, stands in part above one of the spores of the short basidium, *s*, contact is impossible, owing to the protuberancy of the body of the long basidium. *p p* are paraphyses. (2) *b*, the zone of spore-discharge where the basidia are shooting out their spores into an interlamellar space. It is composed of two sub-zones: b_1, a sub-zone of spore-discharge for the long basidia; and b_2, a sub-zone of spore-discharge for the short basidia. In the former, the long basidium, *w*, has already discharged two of its spores, and the other two spores are about to be shot away. At the base of the two remaining spores a water-drop is forming and the lowest drop has attained practically full size. On the opposite side of the Figure a long basidium is about to discharge its last remaining spore. The arrows indicate the direction in which the spores will be shot. For a complete trajectory of a spore, the reader is referred to Fig. 107, p. 252. The short basidia in the sub-zone b_1 have not discharged any of their spores. In the sub-zone b_2 the short basidia only are discharging their spores and the long basidia have vacant sterigmata. On the left of the Figure at *v*, a short basidium has shot away one spore and is about to shoot away the uppermost remaining one. At the base of the spore a water-drop is being excreted. On the opposite side of the Figure a short basidium has already shot away two of its spores, and the other two, since they have water-drops at their bases, will soon be discharged also. The arrows again show the direction in which the spores will travel when they are shot away. The arrows also indicate which spores will be discharged next. On the left-hand side of the Figure, the spores on the long basidium, *w*, will disappear before those on the short basidium, *r*, begin to go. The short basidium, *v*, can now discharge its spores without hindrance, since the spores on the long basidia surrounding it have already disappeared. The order in which eight basidia on the right-hand side of the Figure discharge their spores is shown by the numbers 1 to 8 set opposite the basidia just within the brackets. (3) *c*, the zone of basidia which have shed all their spores; *l*, a long basidium; *s*, a short; *p*, paraphyses. The elements have not yet collapsed. (4) *d*, the zone of autodigestion. Here the cells are becoming indistinct and liquefied. The cell-walls are becoming granular and are disappearing. The projecting end of the long basidium, *l*, is still changed but little or not at all, while the short basidium, *s*, is much shrunken. *p*, a disappearing paraphysis. (5) *e*, the liquid film at the gill-edge containing the products of autodigestion and the spores which, for one reason or other, have failed to be discharged. Toward the top of this zone the outline of the disappearing basidia can still be vaguely traced by rows of granules. Some of the wasted spores held within the liquid film are normal in appearance, while others are below normal size or are colourless or only partially pigmented. The wasted spores form but a small fraction of the whole number. The scale on the right-hand side of the Figure shows the dimensions of every part.

(F). A similar abnormality has already been described in connection with *Panaeolus campanulatus*[1] and *Psalliota campestris*.[2]

Sometimes drops were excreted from the sterigmata in the usual manner and attained their full normal diameter, but spore-discharge

[1] These *Researches*, vol. ii, 1922, p. 308. [2] *Ibid.*, p. 18.



did not ensue. The drop then slowly evaporated. It was observed that spores which lose their drops by evaporation in this way are never shot off their sterigmata, but are eventually dragged down on to the hymenium to which they remain adherent.[1]

Sometimes in the zone of spore-discharge there could be observed certain abnormal basidia. Some of them possessed four undersized spores (Fig. 104, *i*, p. 242), some three spores only, some imperfectly pigmented spores, and some entirely colourless spores. Most of the spores of these basidia failed to be discharged. Both normal-looking and abnormal-looking spores which fail to be discharged are

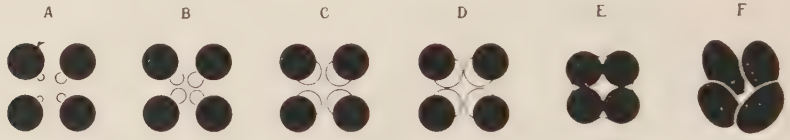


Fig. 106.— *Coprinus sterquilinus.* Abnormal excretion of water-drops. A, four spores of a basidium seen from above. The water-drops excreted from the necks of the sterigmata are protruding towards the axis of the basidium. B, the drops have attained full normal size (the spores at this stage are usually discharged). C, the drops have grown and have stepped up on to the spores. D, the drops are still growing and almost touch one another. E, the drops have fused and the spores have been dragged together by surface tension. F, the large drop has evaporated, the basidium has collapsed, and the spores have been dragged to the surface of the hymenium. The spores are thus wasted, for they do not escape into the air. Magnification, 377.

eventually dragged down to the general level of the hymenium and so become *waste spores.* A dense black line of these waste spores is always to be found along the gill-edge of every gill which is undergoing autodigestion (Figs. 101, 104, 105, pp. 237, 242, 249). The exact manner in which it is formed will be explained more fully a little later, and here it is only necessary to remark that the presence of black spore-lines at the extreme edges of spore-discharging gills not only of *Coprinus sterquilinus,* but also of Coprini generally, is clear evidence that the mechanism for the production and liberation of the spores in the Inaequi-hymeniiferae is by no means always perfect.

The zones of spore-discharge are situated in planes which are almost vertical and their horizontal basidia point forward into

[1] For another illustration showing abnormal drop-excretion in *Coprinus sterquilinus, vide infra,* Part II, chap. i, Fig. 205.

interlamellar spaces. The spores are shot away from their sterigmata in an almost horizontal direction. By making observations with a horizontal microscope in the manner described in Volume I,[1] it was found that, after discharge in still air, a spore travels horizontally for about 0·2 mm. into the interlamellar space, then curves sharply downwards, and then falls vertically. This trajectory resembles that of the spores of other Hymenomycetes. In *Psalliota campestris*, *Coprinus niveus*, *C. curtus*, *Polyporus squamosus*, and many other species with relatively small spores, the length of the horizontal part of the trajectory is only about 0·1 mm., whereas in *Coprinus sterquilinus* and *Amanitopsis vaginata* in which the spores are exceptionally large it is about 0·2 mm., *i.e.* twice as long. The peculiar trajectory traced out by a hymenomycetous spore when shot away from its sterigma I have called a sporabola.[2] Six *Coprinus sterquilinus* sporabolas are shown in Fig. 101, A (p. 237). From a study of this illustration, it will be seen that the spores are not shot sufficiently far to reach even the middle of the interlamellar spaces, although these are represented as being only 0·8 mm. wide.[3] There is therefore no danger of a spore being shot from one gill across the interlamellar space so as to strike and stick to the opposing gill. It will be remembered that a natural-size photograph of a spore-deposit of *Coprinus sterquilinus* is reproduced in Fig. 102 (p. 238). Measurement of the width of the spore-lines of that spore-deposit confirms the statement already made that the spores, before beginning to fall vertically, are shot horizontally into the interlamellar spaces to a distance of only about 0·2 mm.

In Fig. 105 (p. 249) it was not possible to add trajectories of discharged spores, owing to lack of space; but several arrows are there introduced which indicate the directions in which certain spores would begin to travel when shot violently forward from their sterigmata. A piece of the right-hand hymenium of Fig. 105 is reproduced in Fig. 107 with the addition of two complete sporabolas, one for the spore of a long basidium and one for the spore of a short basidium.

[1] These *Researches*, vol. i, 1909, p. 142.

[2] *Ibid.*, p. 185.

[3] This is about the minimum width for an interlamellar space. The interlamellar spaces, as may be seen by reference to the spore-deposit shown in Fig. 102 (p. 238), vary in width from about 1 to 2·5 mm.

The horizontal component of each of these sporabolas is represented as being 0·2 mm. in length,[1] while the form given to the sporabolas is based on a mathematical calculation contained in Volume I.[2]

An examination of Figs. 104 and 105 (pp. 242 and 249) reveals that the zone of spore-discharge is situated at so small a height above the free margin of the gill that a spore, when shot from its sterigma into an interlamellar space, has only to fall a distance of about 0·3 mm. at most to escape from the pileus into the outer

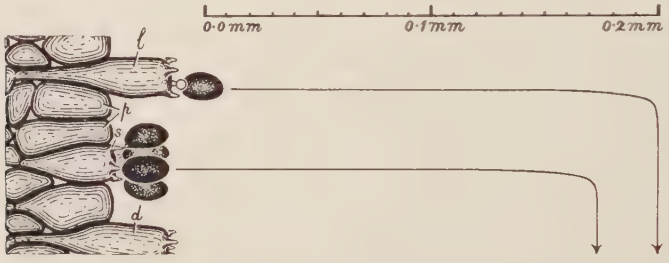


Fig. 107.—*Coprinus sterquilinus.* A vertical section through a gill showing a portion of the hymenium in the zone of spore-discharge (*cf.* Fig. 105, p. 249) and the nature of the trajectories of the spores (*sporabolas*) in still air. The spores are shot out horizontally to a distance of about 0·2 mm.; they then make a sharp curve and fall vertically downwards in the interlamellar space. *l*, a long basidium discharging its spores; *d*, a long basidium which has discharged its spores; *s*, a short basidium about to discharge its spores; *p*, paraphyses. The magnification is shown by the scale.

air beneath, where it may be carried off by the wind. This distance is not much greater than that of the horizontal component of the sporabola, which, as we have learned, has a length of about 0·2 mm. A consideration of these measurements, aided by an inspection of Fig. 101 (p. 237) or Fig. 107 combined with Fig. 105 (p. 249) justifies us in drawing the conclusion that *the spores of Coprinus sterquilinus can escape from a pileus even when the gills are inclined to the vertical at angles of considerable size.*

If the spores in the zone of spore-discharge were to be shot horizontally outwards from the hymenium for a uniform distance of 0·2 mm., or even only 0·15 mm., the gills could be tilted from

[1] *Vide supra.* [2] These *Researches*, vol. i, 1909, pp. 184–191.

the vertical to an angle of 30° and yet all the spores would escape from the pileus. The reader will realise the truth of this statement, if he or she imagines that the three gills shown in Fig. 101, A, (p. 237) have been tilted to the extent postulated, and that the horizontal component of the sporabolas there shown remains unchanged ; for it will be evident that, when the spores shot out from the zone of spore-discharge fall vertically downwards, they will not strike the gill-edges but fall clear of them.

That the spores of *Coprinus sterquilinus* can escape freely from gills which are tilted from the vertical at a considerable angle is also clear from a study of the spore-deposit shown in Fig. 102 (p. 238). That spore-deposit was made by removing an expanded pileus from its stipe and setting it on white paper. The pileus rested on the peripheral parts of its gill-edges ; its gill-planes could not have occupied exactly vertical positions ; and yet, as the double spore-lines prove, spores escaped freely from both edges of each tilted gill and formed excellent spore-deposits on the paper below.

Under natural conditions in the open the gills of *Coprinus sterquilinus* are usually tilted slightly from the vertical ; but, as we have seen, a tilt of 5°, 10°, or even 20°, cannot make any appreciable difference to the freedom with which the spores escape from the pileus.

That under natural conditions the planes of the gills tend to be more or less tilted from the vertical is due to the fact that the gills are subparallel-sided and ageotropic. The gills develop in the first place within the pileus and about the stipe in a series of radial planes ; and the stipe, on becoming negatively geotropic, turns upwards and thus brings the pileus as a whole into an upright position (*cf.* Fig. 75, p. 186, where the stipe is bent owing to its re-action to gravity and the pileus is now upright). The result of all this is that, just before spore-discharge begins, all the gills come to lie in planes which are not absolutely vertical but only more or less so. Since the gills are ageotropic, they cannot adjust their positions in space so as to make them quite vertical. However, there would be no advantage in their making this fine adjustment, even if they could ; for, as we have seen, the mechanism for the production and liberation of the spores is of such a kind that the spores

can readily escape from the pilei even when the gill-planes are tilted in respect to the vertical at angles of appreciable size.

(*c*) *The zone of spore-free surface.* The spore-free zone of the hymenium comes into existence owing to the fact that the zone of spore-discharge moves from below upwards on each gill and thus leaves behind it a zone from which the spores have been discharged. This zone is shown at *c* in Figs. 104 and 105 (pp. 242 and 249). It is parallel to the other zones and has a width of about 0·08 mm. All the elements within it—long basidia *l*, short basidia *s*, and paraphyses *p*—are still living and turgid, and even the sterigmata still retain their original form. In Fig. 105, which shows a cross-section of the zone, the long basidia can readily be distinguished from the short basidia by their difference in length; whilst, in Fig. 104, which shows a surface view of the zone, the long basidia project toward the reader farther than the short ones and therefore can be identified by their more prominent appearance as indicated by heavier shading. Within the spore-free zone, generally speaking, all the basidia have lost their spores and present to the eye nothing but vacant sterigmata. However, owing to inevitable imperfections which introduce themselves into the process of manufacturing and discharging the millions of spores which are developed even on a single gill, one may occasionally observe within the spore-free zone a basidium which has failed to discharge some or all of its spores at the proper time. One such basidium is shown at *i* in Fig. 104: it possesses four undersized colourless spores which, as we shall see, are destined to become waste spores and to join the other waste spores at the gill-edge.

(*d*) *The zone of autodigestion.* The zone of autodigestion lies just below, and parallel to, the zone of spore-free surface and is about 0·04 mm. wide. The spore-free zone is constantly extending above owing to the discharge of spores in the zone of spore-discharge, but it is constantly contracting below owing to the progressive encroachment of the zone of autodigestion. The upper and lower boundaries of the spore-free zone move upwards on a gill at exactly the same rate. Hence the spore-free zone does not appreciably alter in width whilst travelling up a gill from the bottom to the top. This constant width, as we have seen, is about 0·08 mm.

If one places a living gill flat on a glass slide under a cover-glass without any mounting fluid, and if one then watches the spore-free zone with the high power of the microscope, one can observe the autodigestion of the hymenial elements (Fig. 104, zone *d*, p. 242). A cell undergoing autodigestion first loses its turgidity, and then its walls become granular. Soon, the cell-contours become indistinct; and eventually they disappear. The granular particles derived from the cell-walls persist for a time; but they gradually become more and more difficult to trace and, in the end, most of them seem to disappear altogether. It is not improbable that the walls become resolved into a thin mucilage. The basidia are more persistent than the paraphyses, and the projecting long basidia than the non-protuberant short basidia. The part of each basidium to disappear last is the free end crowned with the remains of the sterigmata. This is probably due to the fact that the free end of each basidium does not sink down in the hymenium and become involved in the general autodigestive fluid until some minutes after its last spore has been discharged (*cf.* Fig. 105, *l* and *s* in zone *d*, p. 249). Traces of the basidia, which appear flattened from above downwards, can still be seen at the top of the zone of the products of autodigestion (Fig. 104, zone *e*, p. 242). The zone of autodigestion in Fig. 105 has been drawn to correspond with that in Fig. 104. There is no doubt that the process of autodigestion involves not only the hymenium but also the subhymenium and trama, and the destruction of these inner parts of a gill has therefore been represented in the zone *d* of Fig. 105 (p. 249). The only cells of a gill which escape autodigestion are the waste spores. The zone of autodigestion, whilst moving upwards on a gill, gradually comes to involve every spore which was not successfully discharged when it was in the zone of spore-discharge. One such spore is shown on the left-hand side of Fig. 104 at *m* and another at *n* (p. 242).

When giving an account of the autodigestion of the gills of *Coprinus comatus* in the first volume of this work published in 1909, I expressed the opinion that the phenomenon is brought about by appropriate enzymes liberated from the cell-sap of the dying cells;[1] but, at that time, there was not any experimental proof that such

[1] *Researches on Fungi*, vol. i, 1909, p. 200.

enzymes exist. However, since then, Weir,[1] as already mentioned,[2] has carried out a research upon this question with positive results. According to him the expressed juice of the Coprinus which he used contains an enzyme which can act on the cell-walls and cause them to disappear. My use of the term *autodigestion* to replace the term *deliquescence* has therefore been justified.

(*e*) *The zone of the products of autodigestion.* This zone, which is situated along the gill-edge (zone *e* in Figs. 104 and 105, pp. 242 and 249), varies somewhat in width. For the gill represented in Figs. 104 and 105 it is about 0·06 mm. wide. Above, it passes into the zone of autodigestion. Along its upper edge it contains the granular remains of what were the free ends of the basidia, but lower down all traces of definite elements disappear. Along its lower edge, at the extreme base of the gill, are always a considerable number of spores which for one reason or other have not been discharged properly. These *waste spores* are unable to escape from the liquid film which encloses them and are destined to fall to the ground with the pileus when the whole fruit-body collapses. The zone of the products of autodigestion, like all the other zones, gradually ascends the gill upon which it is situated, passing from the bottom to the top. Throughout the spore-discharge period, its upper edge remains within about 0·25 mm. of the lower edge of the zone of spore-discharge. The liquid, of which the zone is chiefly composed, has been derived from the cells of the hymenium, subhymenium, and trama, which have been broken down in the zone of autodigestion. Doubtless, under natural conditions, a large part of this liquid disappears owing to evaporation, while some of it is sucked along the trama by capillary attraction.[3] In any case it does not accumulate in such a quantity as to swell out the edge of the gill and form an obstacle to the fall of the spores which are shot out from the zone of spore-discharge.

Since the zone of the products of autodigestion continually moves upwards, it gradually comes to envelop all the spores which have not been shot away properly. The spores at *m*, *n*, and *i*, in

[1] J. R. Weir, "Untersuchungen über die Gattung Coprinus," *Flora*, Bd. 103, 1911, p. 271.

[2] P. 231,

[3] *Cf.* pp. 161–163,

Fig. 104 (p. 242) would be enveloped in this way if the zone were to rise 0·15 mm. As spore-discharge proceeds, the number of spores accumulating at the gill-edge gradually increases until so many are collected that they become visible to the naked eye as a thin black line. Many of these waste spores have a perfectly normal appearance, but others a distinctly abnormal one. Some of the latter (*cf.* Figs. 104 and 105, pp. 242 and 249) are undersized, some overgrown, and some partially pigmented or even colourless. As we have seen, the number of spores produced on a pileus may be as many as 100,000,000. It should therefore be no matter for surprise when we find that of this huge total some tens or even hundreds of thousands of spores may be wasted. However, the number of waste spores along the gill-edges of an exhausted pileus is probably less than 5 per cent. of the total number produced ; and certainly it is always small relatively to the number of the spores liberated into the air. In accumulating waste spores upon its gills, *Coprinus sterquilinus* does not differ from other Inaequi-hymeniiferae or from such Aequi-hymeniiferae as *Panaeolus campanulatus*, *Stropharia semiglobata*, and *Psalliota campestris*. My experience goes to show that none of the Hymenomycetes are so perfectly organised that all the spores developed are properly discharged. Several scores of species have come under my observation, but in all of them some waste spores were found upon the hymenium.

Conclusion and Discussion of the Probable Steps in the Evolution of the Inaequi-hymeniiferae.—As already stated in Chapter VI,[1] I have come to the following highly important theoretical conclusion : *in the genus Coprinus, the ripening and discharge of the spores from below upwards on each gill, and the autodigestion from below upwards on each gill, are special arrangements which permit of successful spore-discharge from parallel-sided* (*and subparallel-sided*) *ageotropic gills.* This conclusion fits all the facts that have been brought forward in this Chapter in respect to the production and liberation of spores in *Coprinus sterquilinus* ; for, in this species, it is clear that, owing to the fact that the zone of autodigestion follows hard after the zone of spore-discharge, constantly destroying the spore-free zone from below upwards, the obstacle to the fall of the spores

[1] Chap. vi, p. 119.

which would otherwise be provided by the increase in the spore-free part of the gill is constantly eliminated and the spores can escape from the gills even although the gills are tilted.

In the evolution of the Coprini from the Aequi-hymeniiferae the first change was probably the change of shape of the gills from wedge-shaped in cross-section to parallel-sided or subparallel-sided in cross-section. This permitted of the gills becoming thinner and more closely packed in the young pileus ; but it brought with it the disadvantage that, even with a very precise response to the stimulus of gravity, it became impossible for the gills to take up such strictly vertical positions that the spores after discharge could all escape freely down the interlamellar spaces without striking the sides of the gills. Then, we may imagine, the gills ceased to react to gravity and *pari passu* underwent other important changes. The spores, instead of being produced and liberated from all parts of the gills (every square mm.) simultaneously throughout the spore-discharge period as now occurs in the Aequi-hymeniiferae, came to be produced and liberated in succession from below upwards on each gill. It is not difficult to suppose that the numerous irregular waves of hymenial development which one can observe in the mottled gills of many Aequi-hymeniiferae, *e.g. Panaeolus campanulatus*, became altered in their movements so as to form one grand upward-moving hymenial wave such as we now find on the gill of a Coprinus. The production and liberation of spores is accompanied in all Agaricineae by the emptying of the basidia and paraphyses and by a diminution in the amount of protoplasm, etc., in the hyphae of the subhymenium and trama. The production and liberation of the spores from below upwards on each gill in our evolving Coprinus therefore inevitably brought in its wake the exhaustion of the gills from below upwards ; and the exhaustion of the gills from below upwards was almost as inevitably followed by the death of the gills from below upwards. The death of the gills from below upwards resulted in the liberation of enzymes from the dying cells from below upwards, and this in turn led to the autodigestion of the spore-free portions of the gills from below upwards. The autodigestion of the spore-free portions of each gill from below upwards ensured the progressive elimination of an obstacle which,

if it remained in existence, would prevent the escape of the spores from the pileus. Thus the production and liberation of the spores on each gill from below upwards, together with the autodigestion of each gill from below upwards, made it possible for the spores to be successfully liberated from the pilei even although the gills were more or less parallel-sided and ageotropic. This mechanism for the production and liberation of spores, on account of its success, has persisted in the Coprini until the present day, and it is beautifully exemplified in *Coprinus sterquilinus*.

CHAPTER IX

THE ATRAMENTARIUS SUB-TYPE ILLUSTRATED BY COPRINUS ATRAMENTARIUS

Characters of the Atramentarius Sub-type—Representative Species—*Coprinus atramentarius*. Preliminary Remarks on the Function and Fate of the Cystidia—Distribution and Habitat—General Description of the Fruit-bodies—The Geotropism of the Stipe—The Structure of the Gills—The Process of Spore-discharge—The Function and Fate of the Cystidia during Spore-discharge—The Interlamellar Spaces—Summary concerning the Structure and Function of the Cystidia—The Pilei of *Coprinus atramentarius* and *C. comatus* compared—The Pileus-flesh—Other Coprini belonging to the Atramentarius Sub-type

Characters of the Atramentarius Sub-type.—The Atramentarius Sub-type of fruit-body possesses all the essential characters already described for the Inaequi-hymeniiferous or Coprinus Type: (1) the gills are very thin, (2) the gills are parallel-sided, (3) the gills are not positively geotropic, (4) usually the hymenium on one side of a gill at maturity looks slightly downwards and that on the other side slightly upwards, (5) the spores ripen in succession from below upwards on each gill, (6) the spores are discharged in succession from below upwards on each gill, and (7) autodigestion proceeds from below upwards on each gill and removes those parts of the gills which have become spore-free and which, if they continued in existence, would become mechanical hindrances to the fall of the remaining spores.

The special characters of the Atramentarius Sub-type which enable one to differentiate it from the other Coprinus Sub-types are as follows:

(1) The gills are parallel-sided. They therefore differ from those of the Comatus Sub-type which are subparallel-sided and flanged.

(2) Cystidia are present in large numbers on the faces of the

gills. In this respect we have a marked contrast with the Comatus Sub-type in which the hymenium consists of basidia and paraphyses only.

(3) The interlamellar spaces between the gills, which are required to render possible the free development of the spores on the hymenium, are secured by the cystidia acting as stays or distance-pieces. Each cystidium is attached by its ends to two opposite gills and therefore completely bridges the interlamellar space in which it is developed.

(4) The interlamellar spaces continue to be bridged by the cystidia during the discharge of the spores. This character can be recognised readily in the field and serves to distinguish the Atramentarius Sub-type from all the other Coprinus Sub-types.

(5) The basidia are dimorphic. They are of two lengths, long and short. In this character there is an agreement with most of the other Coprinus Sub-types but not with the Micaceus Sub-type where the basidia are tetramorphic.

Representative Species. — The Atramentarius Sub-type was founded on *Coprinus atramentarius*, but it includes several other species. Among these are the following : *Coprinus picaceus*, *C. stercorarius*, *C. narcoticus*, *C. macrorhizus*, and *C. urticaecola*. All these agree in having their gills compactly welded together into one whole by innumerable bridging cystidia during spore-discharge ; and they all differ, therefore, from the members of the other Coprinus Sub-types, *e.g. Coprinus micaceus*, *C. lagopus*, etc., in which the gills, owing to the expansion of the pileus, become quite free from one another just before spore-discharge begins.

Coprinus atramentarius. Preliminary Remarks on the Function and Fate of the Cystidia.—The central fact to bear in mind in comparing the fruit-bodies of *Coprinus atramentarius* with those of *C. comatus* and *C. sterquilinus* is that in *C. atramentarius* we have what may be called the *cystidial arrangement* for separating the gills, whereas in *C. comatus* and *C. sterquilinus* we have what may be called the *flange arrangement*. The cystidia provide one element, and an important one, in a complicated mechanism which is so constructed that through its operation the spores are efficiently produced and efficiently liberated. As soon as the cystidia are of

no further service, they undergo self-destruction. Correlated with the cystidial arrangement for separating the gills are the following facts : (1) the gills are very thin, (2) the gills are very broad, (3) the gills do not possess flanges, (4) the gills are set close to one another near the pileus-flesh, (5) the gills do not split down their median

FIG. 108.—*Coprinus atramentarius*. Group of medium-sized unexpanded fruit-bodies coming up on soil near the stump of a dead tree. Photographed at Winnipeg. Natural size.

planes from above downwards, and (6) during spore-discharge the interlamellar spaces are very narrow.

In a special paper, published in 1910, I described the manner in which *Coprinus atramentarius* produces and liberates its spores, and I pointed out the rôle played by the cystidia in the working of the fruit-body mechanism.[1] A further study of *C. atramentarius*,

[1] A. H. R. Buller, "The Function and Fate of the Cystidia of *Coprinus atramentarius*, together with some General Remarks on Coprinus Fruit-bodies," *Annals of Botany*, vol. xxiv, 1910, pp. 613–629.

made since 1910, has brought to light the following facts which I had previously overlooked : (1) the dimorphism of the basidia, (2) the accumulation of waste spores on the gill-edges, and (3) the excretion of a water-drop from the hilum of each spore just before spore-discharge. In the account of *C. atramentarius* about to be given in this Chapter there will be incorporated all my observations, both old and new, and, in addition, a new and improved series of illustrations.

Distribution and Habitat.—*Coprinus atramentarius* is one of the largest and commonest species of its genus. It is widely distributed over the earth's surface, and has been found in Europe, North America, Australia, and Kerguelen Island.[1] I, myself, have often seen it in England and Western Canada. The genus Coprinus, according to Hennings,[2] contains about 175 species. Whilst most of them grow upon dung or dunged ground, a few come up on dead stems or wood. *Coprinus atramentarius* belongs to the minority. It is never coprophilous but, so far as my observation has gone, is most frequently found at the sides of partly buried stumps and logs of wood. In England, I have often noticed the fruit-bodies at the foot of gate-posts and the supports of fence rails. At Gimli on Lake Winnipeg, a forest of poplars (*Populus tremuloides* and *P. balsamifera*) was cut down to open up a tract of land for cattle, and the stumps of the trees were left protruding from one to two feet above the surface of the ground. A few years afterwards I visited the spot and found that the fruit-bodies of *Coprinus atramentarius* were coming up about the stumps in great abundance. There were literally hundreds of fruit-body clusters. Upon investigation I found that a number of these clusters were seated directly on root-spurs. It therefore seems probable that the mycelium of *Coprinus atramentarius* is specialised for the destruction of wood. However, the manner in which the fungus passes through its vegetative existence under natural conditions still awaits an exact investigation. At attempt made by myself to

[1] G. Massee, "A Revision of the genus Coprinus," *Ann. of Bot.*, vol. x, 1896, p. 143.

[2] P. Hennings, in Engler u. Prantl, *Die nat. Pflanzenfamilien*, Teil I, Abteil. I**, p. 205.

raise the fungus on sterilised horse dung in the laboratory failed; but Long and Harsch,[1] by inoculating malt agar and parsnip agar, obtained fruit-bodies 25–31 days after inoculation.

Fig. 109.—*Coprinus atramentarius.* A very large unexpanded solitary fruit-body coming up on soil near the stump of a dead tree. Photographed at Winnipeg. Natural size.

General Description of the Fruit-bodies.—The fruit-bodies may occur singly (Fig. 109) but are usually clustered, four or more individuals often being united by the bases of their stipes (Fig. 108). The pilei, before expansion, are usually ovate and more or less plicate, and they vary from about 1 to 3 inches in height; later on, when spore-discharge and autodigestion has begun, they are campanulate and measure from 1 to 3 inches in diameter (Figs. 118, B,

[1] W. H. Long and R. M. Harsch, "Pure Cultures of Wood-Rotting Fungi on Artificial Media," *Journ. of Agric. Research,* vol. xii, 1918, pp. 69 and 72.

and 121, pp. 278 and 284); and, finally, when the destruction of the gills has become far advanced, they are often quite plane and even revolute (Fig. 119, p. 280). Their upper surfaces are silvery grey,

Fig. 110.—*Coprinus atramentarius.* A fruit-body removed from soil near the stump of a dead tree. Just before the beginning of spore-discharge. Photographed at Winnipeg. Natural size.

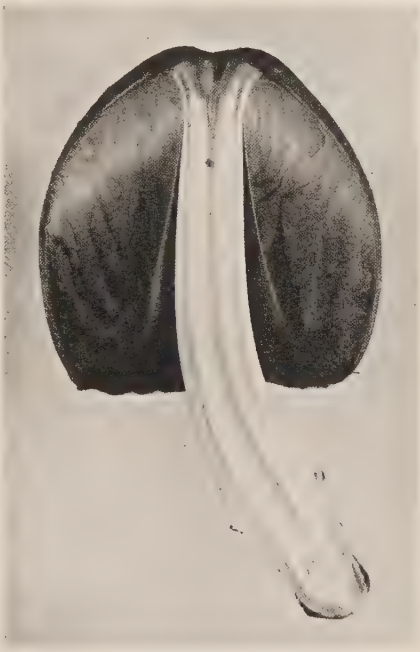


Fig. 111.—*Coprinus atramentarius.* A section of the fruit-body shown in Fig. 110. The spores are ripening from below upwards on the gills. The gills, which are tightly held together by the cystidia, have split down their median planes where the fruit-body was cut in two. The hymenial surfaces are deep black and the tramal surfaces grey. Photographed at Winnipeg. Natural size.

ashen grey, or sometimes brownish in colour; and they are also innato-fibrillose and more or less scaly, especially at the apex (Fig. 109). The flesh is very thin but entire (Figs. 111 and 121, pp. 265 and 284), so that the outer surface of the pileus does not develop the deep sulcations which come into existence in *Coprinus comatus* and *C. sterquilinus* along the backs of the gills (*cf.* Figs. 121 and 77, pp. 284 and 188). The gills, which will soon be described in much greater detail, are broad, thin, ventricose, and closely crowded together. The interlamellar

spaces are traversed by numerous fine cystidia which stretch between and separate adjacent gills. These cystidia can be seen in the field with the naked eye or with a pocket lens. The gills remain connected with one another by the cystidial bridges during autodigestion, even when their upper ends have become entirely free from the stipe. They are destroyed gradually from below upwards by autodigestion, the destruction being just preceded by the liberation of the spores. The spore-discharge period usually lasts from 24 to 48 hours. The spores are oval, $10 \times 5{\cdot}5\ \mu$, and deep brown when seen in water under the microscope. The spore-deposit on white paper is not intensely black as it is in *Coprinus comatus*, *C. sterquilinus*, and *C. niveus*, but very dark brown. The stipe, when fully elongated, varies in length from 2 to 6 inches; and it gradually increases in length as the pileus opens out and the spores are shed. It is 3 to 8 lines thick at the base, tapers slightly upwards, and expands again just beneath the pileus-flesh (Figs. 118 and 119, pp. 278 and 280). It is white, hollow, often fistulose (Fig. 113, p. 269), fibrillose, rather brittle, and at its base there is often a prominent encircling ridge reminding one of a volva and marking the place where the unexpanded pileus was in contact with the stipe (Figs. 109 and 110, pp. 264 and 265).

The Geotropism of the Stipe.—Just before and during pilear expansion the upper part of the stipe of *Coprinus atramentarius*, like the upper part of the stipe in all other Coprini, is very sensitive to the stimulus of gravity. If, during this period, the axis of the stipe just beneath the pileus is not in a vertical position but is set obliquely thereto, the lower side of the stipe, in response to the stimulus of gravity, grows faster than the upper side, with the result that the stipe curves upwards until, as shown in Fig. 111, its axis, just beneath the pileus, is exactly vertical. Under natural conditions in the open, the base of the stipe is always fixed in the ground, while the apex, covered with the pileus, is free to move through the air; and these conditions of fixation, coupled with the geotropic sensitiveness of the stipe, are sufficient to secure that the pileus, before beginning to shed its spores, shall be brought into its normal upright position.

An experiment in which the normal conditions of fixation of the

stipe were reversed was made as follows. A fruit-body, like that shown in Fig. 109 (p. 264), was removed from its substratum and turned through a right angle so that the axis of the stipe and pileus came to occupy a horizontal position instead of a vertical one. The pileus, along with the extreme apex of the stipe, was then fixed with pins so that it could not move, while the stipe-shaft was left with its basal end freely projecting into the air in a horizontal direction. On the next day, it was found that the stipe had curved through a right angle in such a way that its basal end was turned up into the air in the manner shown in Fig. 112. Just as usual, in response to the directive stimulus of gravity, the under side of the stipe had grown faster than the upper; but since, under the conditions of the experiment, the apex of the stipe had been fixed in position instead of the base, of necessity the basal end of the stipe had been turned upwards into the air. An analogous experiment was made by Francis Darwin[1]

FIG. 112.—*Coprinus atramentarius.* The fruit-body was fixed by its pileus so that the axis of the pileus and stipe was horizontal. In response to a geotropic stimulus, the stipe turned its free end up into the air, as here shown. Photographed at Winnipeg. Natural size.

[1] Francis Darwin, "On Geotropism and the Localization of the Sensitive Region," *Annals of Botany*, vol. xiii, 1899, pp. 567–574, Plate XXIX. In Darwin's experiments the root end of the plant, after being turned upwards into the air, was

who fixed the tip of a cotyledon of a grass seedling (Setaria, Sorghum, Phalaris) in a horizontal glass tube, while allowing the rest of the seedling to project horizontally into the air, with the result that the root end of the plant was turned upwards into the air instead of downwards toward the ground.

The Structure of the Gills.—The gills are very thin but also very broad. The breadth varies in different fruit-bodies with the size of the pileus, the largest pilei having the broadest gills. Gills which are 10, 12, or even 15 mm. across are not at all uncommon (Fig. 111, p. 265). In the largest fruit-body which I have seen the gills actually attained a width of 24 mm. (Fig. 113). Few other Hymenomycetes, if any, possess gills of such great width as this. A gill of *Coprinus atramentarius*, although very broad, is yet extraordinarily thin; for the thickness of a large gill, as measured between the outer surface of the two layers of paraphyses, was found to vary from 0·16 mm. near the pileus-flesh to 0·1 mm. near the gill's free edge. Owing to its great breadth and extreme thinness, an isolated gill of *Coprinus atramentarius* is far less rigid and far more flexible than an isolated gill of an aequi-hymeniiferous fungus, such as *Lepiota procera* or *Psalliota campestris*.

The average breadth and thickness and the ratio of breadth to thickness for the gills of large fruit-bodies of *Coprinus atramentarius*, *C. sterquilinus*, and *C. comatus* are given in the following Table.

The Breadth and Thickness of the Gills of Large Fruit-bodies of three Species of Coprinus.

Species.	Average Gill Thickness.	Average Gill Breadth.	Ratio of Gill Breadth to Gill Thickness.
Coprinus atramentarius .	0·13 mm.	12 mm.	92·3 : 1
Coprinus sterquilinus .	0·2 mm.	6 mm.	30·0 : 1
Coprinus comatus .	0·25 mm.	9 mm.	36·0 : 1

From the data given in the Table we may conclude (1) that the gills of *C. atramentarius* are not only absolutely thinner than those

afterwards turned round and round in circles for some days. This was due to the sensitive tip of the cotyledon continuously sending stimuli to the motor region. Whether or not the extreme apex of the stipe is able to behave in a similar manner remains to be investigated.

of *C. sterquilinus* and *C. comatus* but also absolutely broader, and (2) that the ratio of gill-breadth to gill-thickness for *C. atramentarius*

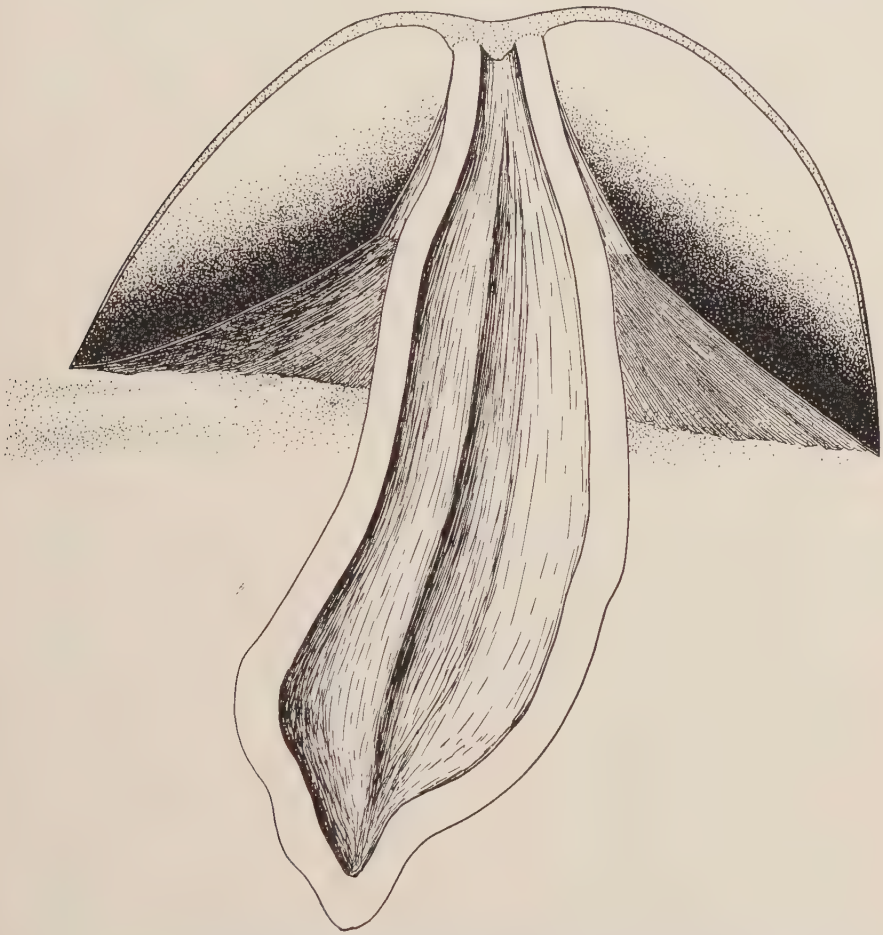


Fig. 113.—*Coprinus atramentarius.* Vertical section through a fruit-body of the maximum size. Spores are ripening on the gills from below upwards and are being discharged from the gill-edges. The gills are extraordinarily broad (2·5 cm.), extremely thin, and closely packed; but the interlamellar spaces are maintained by the cystidia. The spores are shown being carried away by slight air-movement. The number escaping from this fruit-body in all probability exceeded a million a minute. Fruit-body sent to the author by H. T. Güssow from Ottawa. Natural size.

is about three times as great as the ratio for *C. sterquilinus* or *C. comatus.* As a trial proved, an isolated gill of *C. atramentarius* can be flexed by mechanical means much more easily than an

isolated gill of either *C. sterquilinus* or *C. comatus*. This is exactly what might have been expected from a study of the data given in the above Table.

The breadth, thickness, and ratio of breadth to thickness for the gills of the largest fruit-bodies of *Coprinus atramentarius*, *C. sterquilinus*, and *C. comatus* that could be found are given in the following Table.

The Breadth and Thickness of the Gills of the Largest Fruit-bodies of three Species of Coprinus.

Species.	Average Gill Thickness.	Gill Breadth.	Ratio of Gill Breadth to Gill Thickness.
Coprinus atramentarius .	0·13 mm.	24 mm.	184·6 : 1
Coprinus sterquilinus .	0·2 mm.	7 mm.	35·0 : 1
Coprinus comatus .	0·25 mm.	12 mm.	48·0 : 1

From the data given in the last column of the Table we may conclude that, for very large gills, the ratio of breadth to thickness for *Coprinus atramentarius* is about five times the ratio for *C. sterquilinus* and about four times the ratio for *C. comatus*; and a consideration of the data given in both the Tables teaches us that the larger the fruit-bodies, the greater is the increase in the ratio of gill-breadth to gill-thickness for *C. atramentarius* as compared with *C. sterquilinus* and *C. comatus*.

The fact that the gills of *C. atramentarius* are both thinner and broader—and therefore from the mechanical point of view much more delicate—than those of *C. sterquilinus* and *C. comatus* is, I am convinced, directly correlated with the fact that in *C. atramentarius* the gills are kept apart by the cystidial arrangement, whereas in *C. sterquilinus* and *C. comatus* the gills are kept apart by the flange arrangement.

In what follows it must be borne in mind that the gills of *Coprinus atramentarius* are exceedingly thin and yet exceedingly broad, for only by a proper realisation of this fact can the mechanical function of the cystidia be understood.

When two gills of *Coprinus atramentarius* are torn apart, the cystidia can be seen with the naked eye as tiny pellucid processes

projecting from the hymenial surfaces. In a cross-section taken through a series of gills, *i.e.* in the direction *c*—*d* in Fig. 118, A (p. 278), the cystidia can be seen with the naked eye or with the microscope extending across the interlamellar spaces, and they present the appearance shown semi-diagrammatically in Fig. 114 and photographically in Fig. 115. A thin cross-section through two adjacent gills, when highly magnified, reveals that each cystidium consists of a single large cylindrical cell which has a narrowed basal end inserted in the hymenium from which the cystidium has originated and a rounded apical end applied to, and partially embedded in, the hymenium of the opposing gill (Fig. 116, *c*, p. 274).

The cystidia of *Coprinus atramentarius*, like those of many other Hymenomycetes, arise at various depths below the hymenium. The one shown in Fig. 116 (p. 274) had a relatively superficial place of origin, but others were observed which sprang not from the outermost subhymenial cells, but from subhymenial or even tramal cells which were more deeply seated. The cell from which a cystidium springs may become swollen, as shown in Fig. 116, or it may remain cylindrical and be no thicker than the thinnest part of the cystidial stalk.

The apical end of each cystidium is

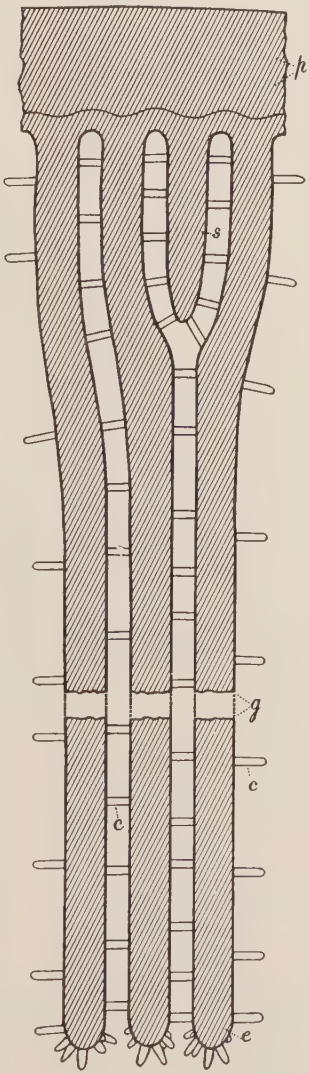


Fig. 114.—*Coprinus atramentarius.* Horizontal section (taken in the direction *c*—*d*, in Fig. 118, A, p. 278) through the pileus-flesh and four gills. The cystidia, *c c*, act as distance-pieces or struts and thus maintain the existence of the interlamellar spaces. *p*, the pileus-flesh; *s*, a short gill not reaching to the stipe; *g*, a gap, this being due to part of the section, equal to about three times its present length, being left out for convenience of illustration: *e*, end of a gill toward the stipe. Magnification about 30.

firmly fixed in the hymenium against which, in the course of its development, it has come to press. If one cuts out a cubical block from such a gill-mass as is shown in Fig. 111 (p. 265), the gill-pieces do not fall apart but remain attached to one another by the cystidia. Owing to the strength of the cystidia crossing the interlamellar spaces and their firm attachment at both ends, one almost invariably breaks any two adjacent gills which one attempts to separate. It is evident that the cystidia lock the gills together laterally and thereby greatly increase the mechanical stability of the whole gill-system.

In such a pileus as is shown in Fig. 110 (p. 265) the weakest radial planes are coincident not with the interlamellar spaces crossed by the cystidia but with the median planes of the gills. Of this fact one may easily obtain experimental proof by breaking the pileus from below upwards and observing the surface of fracture. Let us now examine the fracture-surface which resulted from breaking the pileus of Fig. 110 into two halves and which is shown in Fig. 111. About seven gills have been broken through on each side of the stipe, and their remaining portions are locked together by cystidia. The *black* streaks and patches alone are the hymenial spore-producing surfaces of the gills, and they are black owing to their being covered with black spores held upon the sterigmata. The lighter *grey* areas, which are much more extensive than the black, are tramal surfaces. Hence it is clear that, when the pileus was split into two halves, the part of each gill which happened to lie in the plane of fracture split for the most part down its median plane so that its two halves became separated from one another, one half remaining attached to one pileal hemisphere and the other half to the other pileal hemisphere.

When two adjacent gills are forced apart by mechanical pressure suitably applied, most of the cystidia break away from one of the gills at their apical ends and remain attached to the other gill by their basal ends (Fig. 122, *i*, p. 287), while certain others break away from one of the gills by their basal ends and remain attached to the other gill by their apical ends (Fig. 122, *j*). Worthington Smith[1] doubtless saw cystidia projecting from a gill with their basal ends pointing outwards, for his illustration shows one upside down.

[1] W. G. Smith, "Cystidia in the Mushroom Tribe," *Grevillea*, vol. x, 1881, p. 78.

He evidently thought that the narrowed end of each cystidium was the apical end and that, when the spores were being discharged, it projected freely into an interlamellar space. It may well have been this error which suggested to him that a cystidium is really like a flask in function, that the wall closing the end of the neck acts as a stopper which drops out when the cystidium is ripe, and that through

FIG. 115.—*Coprinus atramentarius*. Photomicrograph of a transverse section through several gills, showing the cystidia crossing the interlamellar spaces, and separating the parallel-sided gills. Magnification, 61.

the neck the cystidial contents escape. His supposed discoveries that the cystidia produce spermatozoids [1] and fall from the gills with the spores [2] were simply further errors suggested by his imagination and involved with his fanciful theory that the cystidia are antheridia and that their spermatozoids fertilise the spores, which he regarded as female gametes. The reason why the base of a cystidium

[1] W. G. Smith, "Reproduction in *Coprinus radiatus*," *Grevillea*, vol. iv, 1875, p. 62.

[2] W. G. Smith, *loc. cit.* On p. 60 he says: "The spores naturally fall to the earth, and with them the cystidia, and it is upon the moist earth that fertilisation is generally carried out."

is narrowed is readily understood when one remembers that it is necessary for the developing cystidium to remain in connection with one of the relatively small subhymenial or tramal cells. The general shape of a cystidium is very much like that of a strut or distance-piece such as one sees used for preventing the collapse of the sides of deep trenches made in the ground. The narrowed end at the base and the blunt rounded end at the apex are well suited to prevent the cystidium from being pushed through the hymenium of either gill by gill-pressure.

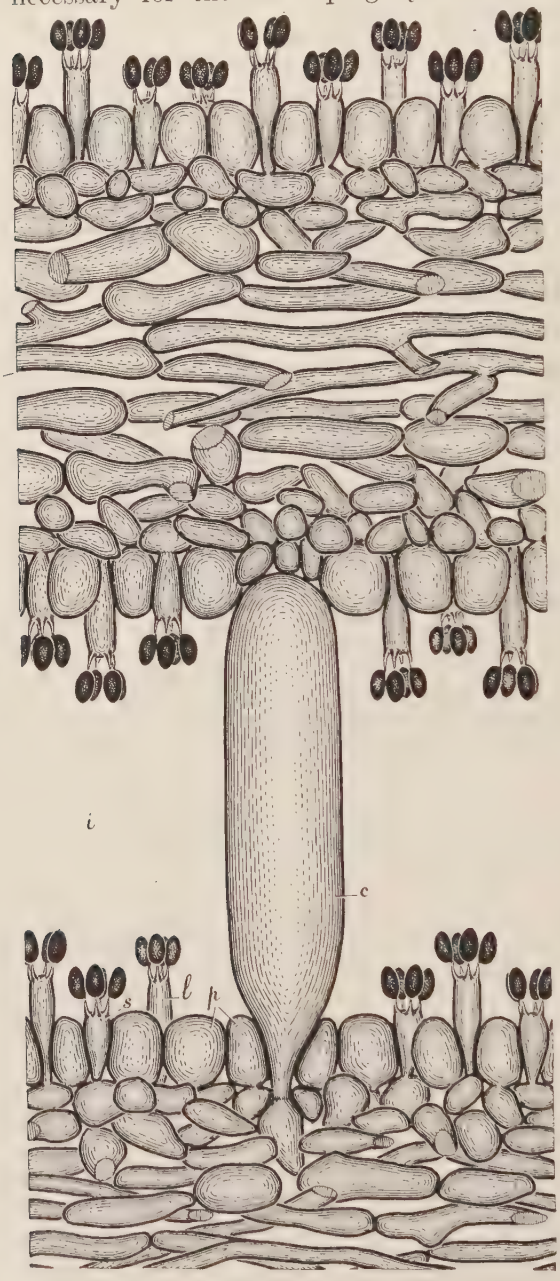


FIG. 116.—*Coprinus atramentarius*. Cross-section showing a cystidium, *c*, stretching across an interlamellar space, *i*, between two gills and thus separating two opposing hymenial surfaces. The dimorphism of the basidia is also evident; *l*, a long basidium; *s*, a short basidium; *p*, paraphyses. Magnification, 440.

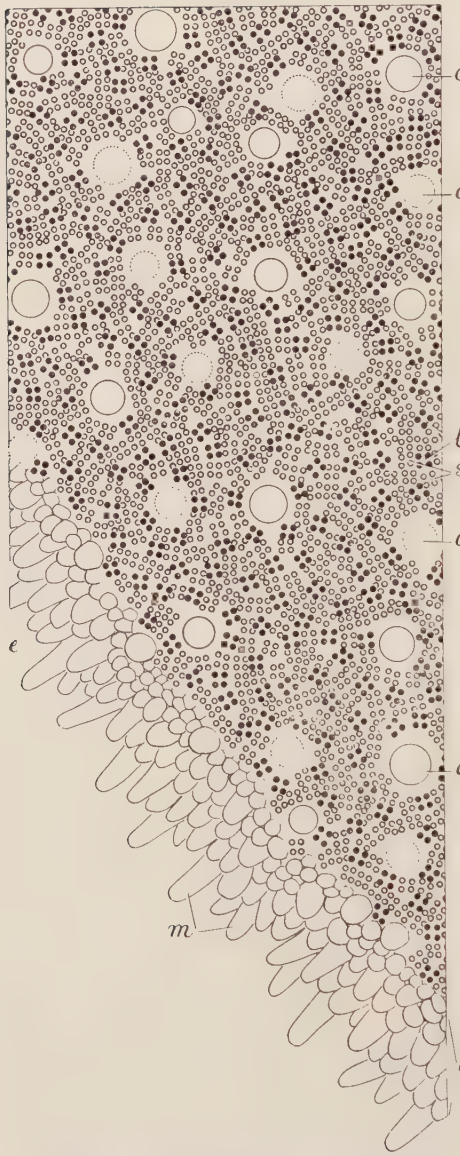
The cystidia of *Coprinus atramentarius* are some of the largest cells found

in fungi; they are usually 0·12 to 0·17 mm. long and 0·02 to 0·03 mm. wide. The largest I have seen were 0·21 mm. long and 0·03 mm. wide. They attain their maximum diameter at a very early stage in the development of the hymenium—long before the basidia begin to produce sterigmata—and then they grow in length for some hours. I have observed that in *Coprinus niveus* the cystidia elongate by means of apical growth and, doubtless, the cystidia of *Coprinus atramentarius* elongate in the same manner. A cystidium, when very young, is short and provided with a large amount of protoplasm; but, as it grows in length, its living substance gradually becomes used up. A full-grown cystidium contains a slight accumulation of protoplasm at both its apical and basal ends, a very thin protoplasmic layer pressed against the wall of its cylindrical shaft, and a large central vacuole filled with clear and colourless cell-sap. The cell-wall is very thin and quite smooth. A cystidium collapses as soon as it has been killed. It is therefore evident that the rigidity which it exhibits when connecting two gills in a living pileus is due to its turgidity.

The distribution of the cystidia over the surface of the gills is fairly uniform, as is shown in Fig. 117. It was estimated, after *camera-lucida* drawings had been made, that from 30 to 60 cystidia are present on each square millimetre of the hymenium. The cystidia are sufficiently numerous to make it impossible for the gills to sag laterally and thus to touch one another with their hymenial surfaces.

As shown in Fig. 114 (p. 271), there are no cystidia projecting radially into the interlamellar spaces where the gills join with the pileus-flesh, and having free ends which point towards the stipe. In view of what I believe to be the function of the cystidia this absence is significant.

When one considers the shape, size, structure, position, and number of the cystidia in the interlamellar spaces of a young pileus and also the extraordinary thinness, breadth, and mechanical weakness of the gills when detached from the cystidia, one is justified in coming to the following conclusion: *the cystidia are unicellular organs which function by holding the gills apart and thus maintaining interlamellar spaces in which the spores can develop without mechanical hindrance.*



The gill-edges, which abut upon the stipe when the pileus is unexpanded, are covered with swollen sterile cells, smaller than, but resembling in general characters, the cystidia which have just been described (Fig. 114, *e*, p. 271, and Fig. 117, *e*). To distinguish the cystidia at the gill-edges from those on the gill-sides, we may refer to the former as *cheilocystidia* and the latter as *pleurocystidia*.[1]

In *Coprinus comatus* and *C. sterquilinus*, as we have seen, there are flanges at the gill-edges. In *Coprinus atramentarius*, on the other hand, there are no such flanges (*vide* Fig. 114, *e*, p. 271).

The hymenium of *Cop-*

Fig. 117.—*Coprinus atramentarius.* Surface view of a piece of gill, 1·04 mm. long and 0·4 mm. wide, taken from the region *r* in Fig. 118, A. To show the distribution of the cystidia and basidia just before the beginning of spore-discharge and autodigestion. *e*—*e*, the oblique free inner edge of the gill bearing marginal cystidia, *m*; *c*, cystidia projecting from the gill; *a a*, places where cystidia from the adjacent gill were in contact with the hymenial surface before the gills were torn apart. Between the cystidia are shown the spores of the basidia. The spores were all black, but for diagrammatic clearness the spores of the long basidia only, *l*, have been made black, whilst those of the short basidia, *s*, have been left unshaded. Magnification, 147.

[1] *Cf.* these *Researches*, vol. ii, 1922, pp. 248, 324; also vol. iii, pp. 52–53.

rinus atramentarius consists of three kinds of elements: basidia, paraphyses, and cystidia. The basidia have the usual coprinoid structure and arrangement, *i.e.* they are dimorphic and crowded, so that the spores of the long basidia often stand in part over the spores of the short basidia (Fig. 120, zone *a*—*a*, p. 281). From three to five paraphyses surround each basidium and thus separate adjacent basidia from one another. The degree of crowding of the basidia varies somewhat according to the extent to which the gills have become stretched during the opening of the pileus. While the gills are stretching, the paraphyses, which alone are the elastic elements of the hymenium, expand; and thus the basidia become more and more separated from one another. The distribution of the spores is shown in Fig. 117 (p. 276). The absence of spores around the cystidia is due to the fact that every cystidium is encircled by paraphyses. The distribution of the cystidia has been sufficiently discussed already. The general significance of the arrangement and structure of the basidia and paraphyses is exactly the same as in *Coprinus comatus* and *C. sterquilinus*.[1]

The spores of *Coprinus atramentarius* ripen from below upwards on each gill; and, as they ripen, they turn dark brown. In the mass, therefore, they appear to be almost black. Hence it is that the gills are black at their bases first and that the blackening process progresses from below upwards on each gill. Stages in the blackening of the gills are shown in Figs. 111 (p. 265), 113 (p. 269), and 118, A, B.

The Process of Spore-discharge.—When the pileus has become expanded to the extent shown by the broken gill-lines at B in Fig. 118, the process of spore-discharge begins. The first spores to be discharged are those which are situated in the narrow zone which extends along both sides of the extreme lower margin of each gill (Figs. 118, B, *s*, and 117, p. 276). As in *Coprinus sterquilinus*, the long basidia in this zone discharge their spores slightly in advance of the short. The zone of spore-discharge moves slowly up both sides of each gill from the bottom to the top.

As soon as a narrow zone (somewhat less than 0·25 mm. wide) along the bottom of each gill has become spore-free owing to spore-

[1] *Cf.* pp. 156–157, 206.

discharge the process of autodigestion begins. The cells in the spore-free zone, as well as those in the sterile marginal band shown

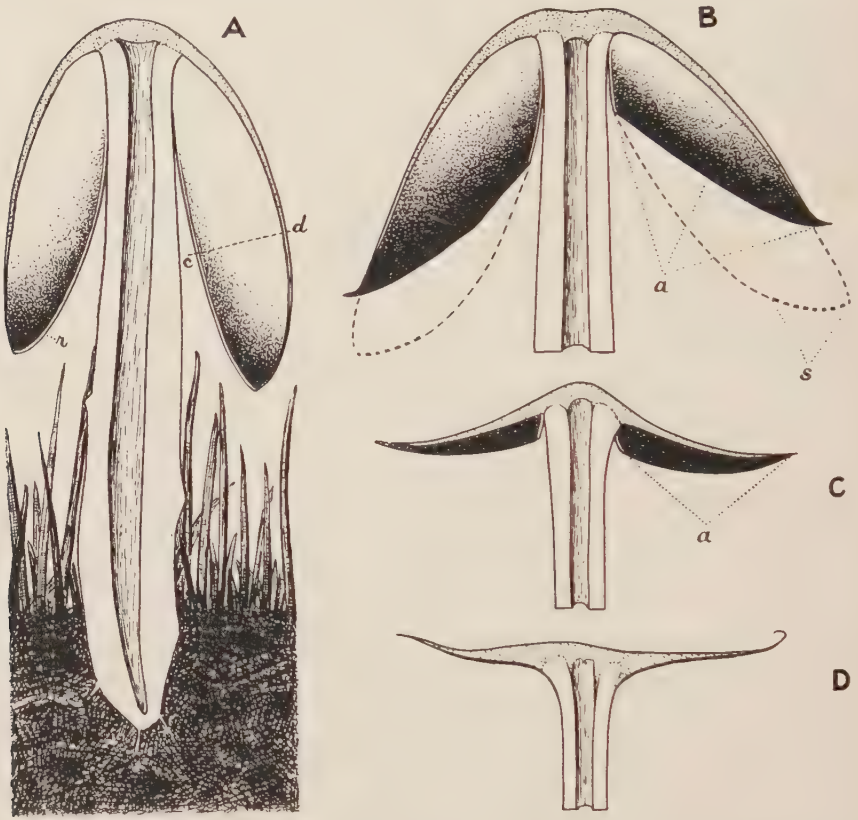


FIG. 118.—*Coprinus atramentarius*. Stages in the development of a fruit-body as shown by vertical sections. A, section of a young fruit-body in which the pileus is just opening out. The spores, as is indicated by the shading, are ripening from below upwards on the gills. Spore-discharge and autodigestion have not yet begun. The flesh is thin and the gills very broad. *c—d*, the direction in which the section shown in Fig. 114 was cut; *r*, the region from which the piece of gill sketched in Fig. 117 was taken. B, section of another and older fruit-body. The spores are still ripening from below upwards on the gills. Spores are being shed and autodigestion is taking place along the gill-edge at *a*. The broken lines show the shape and extent of the gills at the moment autodigestion began; *s*, lower edge of the gill where spore-discharge and subsequently autodigestion first became active. C, section of a still older fruit-body, the pileus of which has become helmet-shaped through expansion. The gills have now become reduced by autodigestion to about one quarter of their original size; *a*, the edge of the gill where spore-liberation and autodigestion are still in progress. D, section of a fruit-body in the last stage of its development when spore-discharge has ceased. The gills have now become reduced to mere ridges. The central part of the pileus-flesh still crowns the stipe. Natural size.

at *e* in Fig. 117 (p. 276), break down, become fluid, and disappear. Doubtless, the destruction of the cell-walls is due to enzymes liberated by the dying cells. The zone of autodigestion, which has come into existence in the manner just explained, then gradually ascends and destroys each gill from below upwards. The zone of autodigestion follows hard after the zone of spore-discharge but never invades it, for it restricts its destruction to the zone which has become free from spores.

After autodigestion has begun, five zones can be distinguished in succession from above downwards on each gill within half a millimetre of, and parallel to, its edge :

(1) a zone of basidia with ripe spores,
(2) a zone of spore-discharge,
(3) a zone of spore-free surface,
(4) a zone of autodigestion, and
(5) a zone containing the products of autodigestion at the gill-edge.

These five zones, which are all shown in Fig. 120 (p. 281), keep their relative distances unaltered ; and they gradually move upwards so that, in the course of about 48 hours, they involve the whole of each gill. The gradual destruction of the gills from below upwards and their disappearance is shown in the series of drawings A, B, C, and D, reproduced in Fig. 118 (p. 278).

The pileus shown in vertical section in Fig. 119 has opened out so that its upper surface is now almost flat ; its rim is slightly revolute ; and its gills have become reduced by autodigestion from below upwards to less than one quarter of their original size. The spores being discharged from the zones of spore-discharge at the gill-edges cannot be distinguished owing to their excessively minute size but, at the time the photograph was taken, some hundreds of thousands of them, and possibly more than a million, must have been carried away by the wind from beneath the pileus every minute. The spore-stream of an active pileus is represented diagrammatically in Fig. 113 (p. 269).

The discharge of the spores from the individual basidia of *Coprinus atramentarius* takes place in the same manner as that already described for other Coprini and the Hymenomycetes generally :

(1) the four spores of each basidium are violently propelled more or less perpendicularly outwards from the hymenium into the adjacent interlamellar space ; (2) they leave the sterigmata one by one and are not all discharged together ; and (3) the time elapsing between the shooting away of the first and last of the four spores of a basidium is usually from one to ten minutes. The discharges can be observed without difficulty if one lays a piece of a gill like that shown in Fig. 120 in a closed compressor cell and looks down upon it

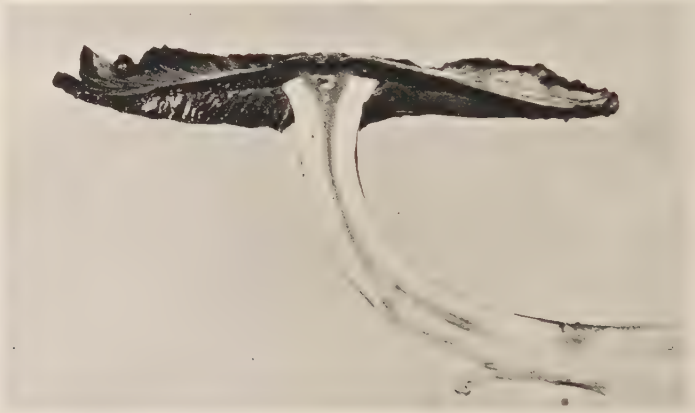


Fig. 119.—*Coprinus atramentarius.* An expanded fruit-body in an advanced state of autodigestion, shedding spores. The gills have become reduced to about one-seventh of their original size. Photographed at Winnipeg. Natural size.

from above with the microscope.[1] The spores can then be seen leaving the basidia in the zone of spore-discharge. Some of the basidia will be found to have four spores upon them, some three, some two, some one, and some none at all.

Just before the spore is to be discharged, a small drop of water is excreted from the hilum of the subjacent sterigma, as in all other Hymenomycetes (Fig. 120, *h*, *i*). The time which elapses from the beginning of the excretion of each drop to the discharge of the spore which carries it away has a duration of from 5 to 10 seconds. Normally in Hymenomycetes, and I believe in *Coprinus atramentarius*, when a spore has been shot away, the end of the sterigma

[1] The compressor cell is employed to protect the gill from too rapid loss of moisture.

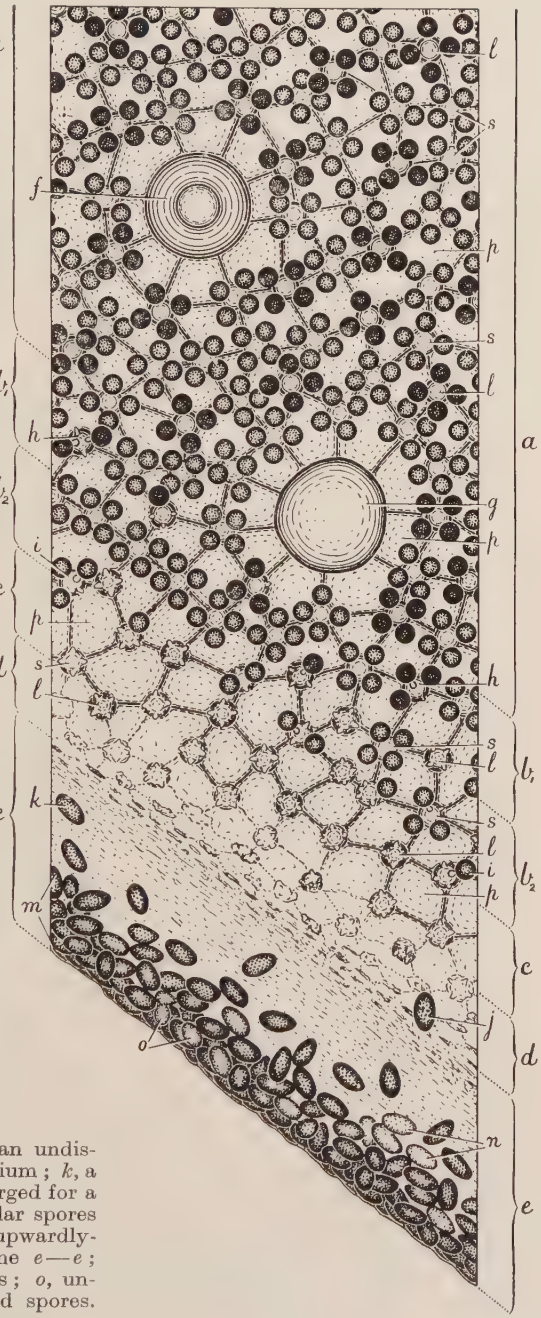


FIG. 120.—*Coprinus atramentarius*. The general appearance of part of a gill-surface, including the gill-edge, after spore-discharge and autodigestion have begun (*cf.* Fig. 118, B, p. 278). There are five zones running parallel to the oblique gill-edge: (1) *a*—*a*, zone of basidia with ripe spores; *l l*, long basidia; *s s*, short basidia; *p p*, paraphyses; *f*, a cystidium undergoing autodigestion, its end is still unmelted; *g*, a cystidium reduced to a drop of fluid. (2) b_1b_2—b_1b_2, the zone of basidia discharging spores into an interlamellar space. This is divided into two subzones: b_1—b_1, the zone of spore-discharge of the long basidia, and b_2—b_2, the zone of spore-discharge of the short basidia. The spores are shot off their sterigmata successively so that in both subzones some basidia have three spores on them, some two, some one, whilst some have lost, or are about to lose, them all; *l*, a long basidium; *s*, a short basidium; *h h*, basidia in the zone b_1—b_1 in which some of the spores have a drop of water at their hila and are about to be discharged; *i i*, similar basidia in the zone b_2—b_2. (3) *c*—*c*, the zone of basidia which have discharged all their spores; *l*, a long basidium; *s*, a short basidium; *p*, a paraphysis. (4) *d*—*d*, the zone of autodigestion. The basidia and paraphyses are becoming indistinct and gradually liquefied. (5) *e*—*e*, the liquid film at the gill-edge containing the products of autodigestion and also the waste spores which failed to be discharged into the air; *j*, an undischarged spore, lying by its basidium; *k*, a spore which has been undischarged for a longer time; *m*, a mass of similar spores which have been collected in the upwardly-moving liquid film of the zone *e*—*e*; *n*, imperfectly pigmented spores; *o*, under-sized and feebly pigmented spores. Magnification, 440.

from which the spore has been shot remains naked and quite devoid of any trace of a liquid drop. However, an exception to this general rule was observed in a fruit-body of *Coprinus atramentarius* which had been kept in a very moist condition under a bell-jar during a whole night. A gill was taken from this fruit-body and placed in a closed compressor cell, and the zone of spore-discharge was watched with the microscope in the usual way. It was then seen that a number of sterigmata, directly after discharging their spores, began to excrete liquid drops at their apices. These drops continued to grow for about 10 seconds and then their growth entirely ceased. This exceptional drop-excretion from the ends of sterigmata which have just shot away their spores may have some bearing on the explanation of the mechanism of spore-discharge.

In the first volume of these *Researches*, I showed that the spores of *Psalliota campestris*, *Marasmius oreades*, and *Polyporus squamosus* are shot forward in almost a straight line into the interlamellar spaces to a distance of about 0·1 mm. and that the horizontal motion is very rapidly brought to an end owing to the resistance of the air.[1] In still air, in consequence of this resistance and of the attraction of gravitation, a spore, when nearing the end of its horizontal flight, describes a sharp curve and falls vertically downwards. I have called this peculiar trajectory a *sporabola*.[2] By using methods already described,[3] I have proved to myself that the spores of *Coprinus atramentarius* have a trajectory similar to that of the Mushroom and other Hymenomycetes, and that the horizontal distance of discharge is of the order of 0·05 mm. Unfortunately, an exact determination of this distance could not be made owing to the limited time during which the living fruit-bodies were at my disposal. On the assumption that 0·05 mm. is the average horizontal distance of discharge of spores shot out perpendicularly from the hymenium, I have indicated the sporabolas of a few spores in the zone of spore-discharge in the semi-diagrammatic drawing reproduced in Fig. 122 (p. 287). It is certain that the spores are shot forward to such a distance from the basidia that they fall down somewhere near the middle of the interlamellar

[1] These *Researches*, vol. i, 1909, pp. 142, 185.
[2] *Ibid.*, p. 185. [3] *Ibid.*, pp. 133–147.

spaces. Thus violent spore-discharge, here as elsewhere, prevents the adhesive spores from coming into contact with one another or with the gill-surfaces during their fall.

The zone of spore-discharge, as in *Coprinus sterquilinus*, is divided into two sub-zones : (1) a higher sub-zone where alone the long basidia are discharging their spores, and (2) a lower sub-zone where alone the short basidia are discharging their spores. The discharge of the spores of the long basidia before those of the short is shown in the zone b_1–b_1 of Fig. 120 (p. 281) and in the zone *d* of Fig. 122 (p. 287).

The zone of spore-free surface and the zone of autodigestion (*c* and *d* in Fig. 120, p. 281 ; *e* and *f* in Fig. 122, p. 287) are very narrow and are similar to those already described in detail for *Coprinus sterquilinus*. The zone of the products of autodigestion contains the liquid products resulting from the breaking down of the hymenial, subhymenial, and tramal cells, and occupies the free margin of each gill. In dry weather, the water in the films at the gill-edges evaporates sufficiently rapidly to prevent the accumulation of drops. However, under moist weather conditions, inky drops may come to hang from the margin of the pileus, just as in *Coprinus comatus*. A photograph of a pileus to the rim of which inky drops are hanging is reproduced in Fig. 121. Inky drops are always formed when a pileus is caused to liberate its spores and undergo autodigestion in a damp-chamber. Under natural conditions, such as those to which the fruit-bodies shown in Fig. 121 were subjected, the dark drops are only to be found at the more or less upturned pileus-rim. Since this rim is composed merely of flesh and of exhausted gill-remains, and therefore does not liberate any spores, it is clear that the drops hanging from it are out of the path of the stream of spores which the wind is carrying away from beneath the pileus. A few spores may be caught in each drop but, under natural conditions, the dark colour of the drops is due, as in *Coprinus sterquilinus*, to a brown pigment which develops in the products of autodigestion as a result of enzyme action.

Upon the gills of a large fruit-body of *Coprinus atramentarius* there are several thousands of millions of spores. A certain small percentage of them, as in *Coprinus sterquilinus* and all other

Agaricineae, fails to be discharged. These *waste spores* are gradually overtaken by the upward-rising liquid film containing the products

Fig. 121.—*Coprinus atramentarius.* Fruit-bodies coming up on soil in a garden, shedding spores and undergoing autodigestion. Drops of a dark fluid hang from the exhausted rim of the pileus. Photographed at Swansea, Wales, by H. R. Wakefield. A little less than natural size.

of autodigestion, with the result that they accumulate at the gill-edges in large numbers (Figs. 120, *e*, and 122, *g*, pp. 281 and 287). So numerous are they that, along the extreme edge of each gill, they form a thin black line which can be seen with the naked eye. Two

spores which failed to be discharged and which have been dragged down on to the hymenium by the collapse of their basidia are shown at *j* and *k* in Fig. 120 (p. 281). These two spores are about to be added to the other waste spores which, owing to the upward movement of the liquid film at the gill-edge, have become crowded together. An examination of the spores held by the liquid film reveals that, while some of them are of very small size (*o*) and others of full size but imperfectly pigmented (*n*), the majority have a normal appearance (*m*). One and all appear to resist the action of the enzymes which, we may suppose, are present in the fluid at the gill-edge.

The Function and Fate of the Cystidia during Spore-discharge.— The cystidia are so large and numerous that, if they persisted until the zone of autodigestion reached them, they would form a serious hindrance to the escape of the spores from between the gills : they would block up so large a proportion of the interlamellar spaces that a great many spores would settle upon them, adhere to them, and thus never reach the outer air. But a beautiful arrangement is provided which makes all this impossible : *the cystidia do not undergo autodigestion at the same time as the basidia and paraphyses in their immediate vicinity but a short time previously thereto ;* they destroy themselves in succession from below upwards on each gill, and each one disappears a few minutes before the basidia in its immediate neighbourhood come to be involved in the upwardly progressing zone of spore-discharge.

The semi-diagrammatic drawing, Fig. 122, shows what was made out by a study of gill-sections cut from blocks of living gills with a hand-razor. The drawing is supposed to represent a section cut in a vertical plane through three gills. From above downwards, the following seven zones can be distinguished :

(*a*) a zone with less ripe spores in which the cystidia are fully turgid and are functioning as distance-pieces to keep the gills apart,

(*b*) a zone with riper spores in which the cystidia are disappearing owing to autodigestion,

(*c*) a zone of ripe spores from which the cystidia have already disappeared,

(*d*) a zone of spore-discharge,

(*e*) a zone which is spore-free,

(*f*) a zone of autodigestion and, finally, at the gill-edge,

(*g*) a liquid film containing the products of autodigestion and the wasted spores.

It is clear from an inspection of the Figure that, *owing to their early autodigestion, the cystidia cannot possibly hinder the fall of the spores and prevent their escape from between the gills.*

If one wishes to prove to one's self that the cystidia would form a serious hindrance to the fall of the spores unless they were removed in the manner described above, one has only to proceed as follows : turn a pileus which is shedding its spores, or a portion of it like that represented in Fig. 122, upside down and then observe the cystidia crossing the interlamellar spaces with the low power of the microscope. One then sees that the spores continue to be shot out from the basidia as under normal conditions but that, on falling down the interlamellar spaces, they strike upon the cystidia, adhere to them, and soon come to cover them with a dense dark spore-deposit.

Since the cystidia of *Coprinus atramentarius* are essential constituents of the fruit-body mechanism in that they alone prevent the very thin, very broad, and very flexible gills from coming into contact with one another, it is not surprising that we should find that they are retained between the gills as long as is possible without their becoming hindrances to the fall of the spores. They are removed only just in time to prevent them from ever extending between those parts of the gills where spore-discharge is taking place. The cystidia-free portions of the gills which hang downwards (Fig. 122, zones *c*, *d*, *e*, *f*, and *g*), collectively, are only about 0·25 mm. in depth. They are kept apart by means of the cystidia above them, so that there is no danger of the interlamellar spaces between them becoming reduced in width. As a matter of fact, the interlamellar spaces in the regions of spore-discharge are distinctly broadened out owing to a slight contraction of those parts of the gills which are undergoing autodigestion (*cf.* zones *d*, *e*, *f*, and *g*).

The fate of the cystidia was found out by studying sections about 1 mm. thick cut transversely through the gills. Some of the sections were cut in planes which were transverse to the gill-edges

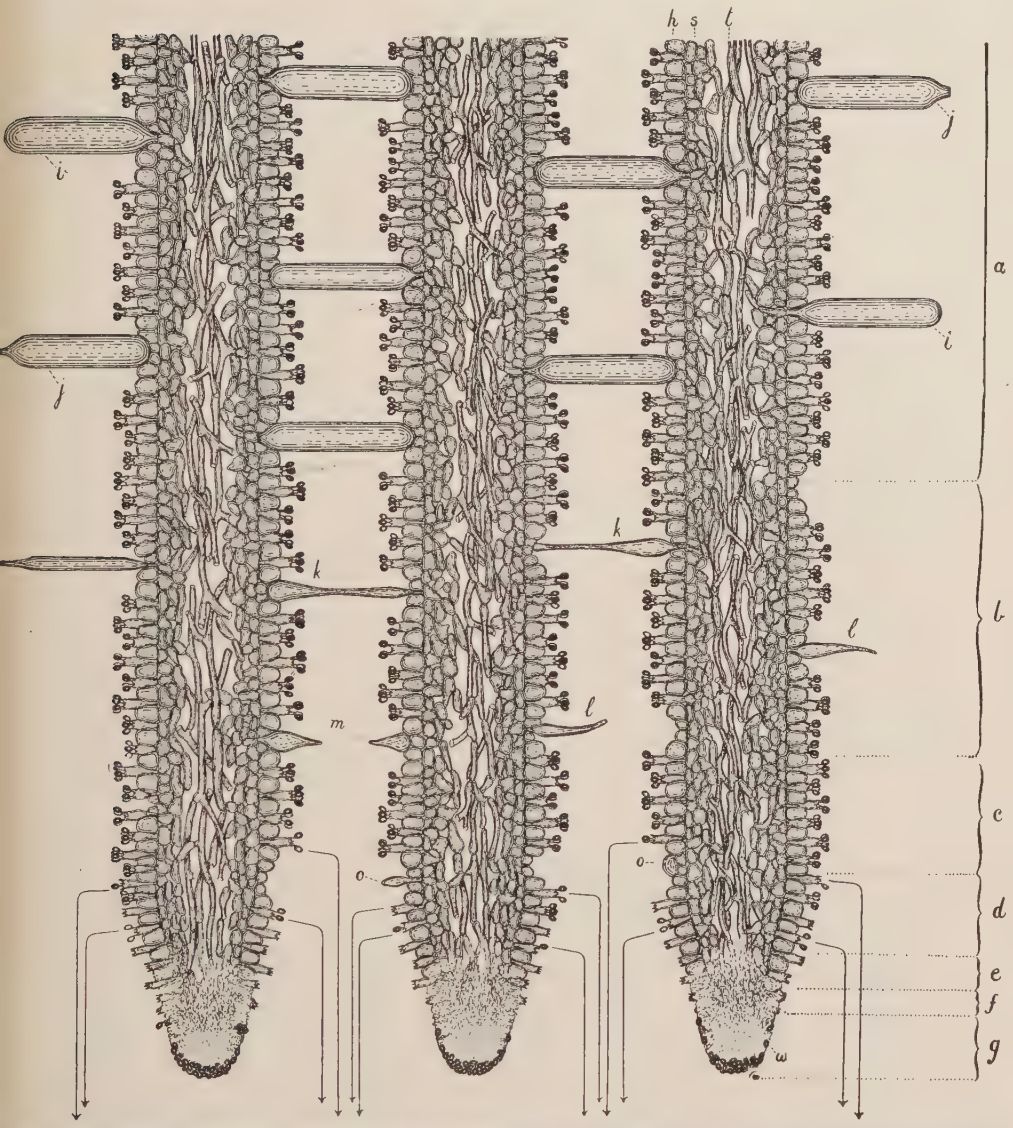


FIG. 122.—*Coprinus atramentarius*. Semi-diagrammatic section taken in a perpendicular plane through three gills during spore-scharge and autodigestion (*cf.* Fig. 118, B and C, p. 278). Seven zones can be distinguished. (1) *a*, zone in which the cystidia are lly turgid and, by functioning as stays or distance-pieces, are holding the gills apart. The basidia bear spores which are nearly ripe.) *b*, zone in which the cystidia are disappearing through autodigestion. The spores are ready for discharge. (3) *c*, zone of ripest ores from which the cystidia have already disappeared. (4) *d*, zone of spore-discharge where cystidia are absent. Spores are being ot out into the interlamellar spaces and their sporabolas are represented by arrows. (5) *e*, the spore-free zone. (6) *f*, the zone of todigestion where basidia and paraphyses are being destroyed. (7) *g*, the liquid film at the gill-edge containing the products of todigestion and undischarged spores. *h*, the hymenial layer made up of basidia, paraphyses, and cystidia; *s*, the subhymenial cells; the trama. *i i*, cystidia which have been torn from the opposite gills by their rounded apical ends. *j j*, cystidia which have been torn om the opposite gills in which they originated so that they are now attached by their apical ends: their contracted bottle-neck-like sal ends are free. *k k*, cystidia which have become very slender owing to autodigestion, but which are still attached to both gills. cystidia which are being withdrawn to the gill-sides. *m*, a cystidium which has snapped into two parts. *o o*, remains of cystidia. erywhere the basidia are seen to be dimorphic, long and short ones alternating with one another. Magnification, 136.

and therefore resembled that shown in Fig. 122 (p. 287), whilst others were cut parallel to the gill-edges and in such a way that one side of each section was made up of the free autodigesting edges of several gills. In all cases, the sections, immediately after being cut, were placed in a compressor cell in order to prevent undue transpiration. Where a section included free gill-edges, it was turned upside down so that the edges looked upwards. On observing the sections with the low power of the microscope, it was possible not only to see the cystidia stretching across the interlamellar spaces but also to watch their disappearance.

With sections such as those just described one can make the following observations. At about 0·5 mm. above the extreme edge of each gill the cystidia, as indicated by a slight diminution in diameter, are already beginning to disappear. Nearer to the autodigesting gill-edge, they have become very much reduced in diameter (Fig. 122, p. 287, *k k*, in zone *b*). Still nearer to the gill-edge, they have become detached from one gill and partly withdrawn to the other gill, usually to the one from which they originated (*l l*, in zone *b*), or, as sometimes happens, they have become broken in two either in the middle or near one end (*m* in zone *b*). The cystidia which are just above the zone of spore-discharge have become reduced to practically nothing, so that it is difficult or impossible to detect any trace of them.

The disappearance of individual cystidia was observed in a considerable number of instances. Stages in the autodigestion of six cystidia are shown in Fig. 123. The time which elapsed between the initial shrinking of a cystidium and its total disappearance was found to be less than half an hour. It took ten minutes for the cystidium represented at C to pass from the fully turgid condition shown at *a* to the final stage shown at *d*, and the break in the cystidium represented at D occurred fourteen minutes after the initial thinning was detected.

One may ask: what becomes of the fluid which is liberated from a cystidium during its autodigestion? Doubtless, part of it simply evaporates; but it is probable that some of it is drawn by capillarity into the interhyphal spaces of the subhymenium and trama. If there were no absorption of this kind, it seems likely

that, whenever owing to saturation of the air with water-vapour evaporation was brought to a standstill, a cystidium on breaking down would form a large drop which would tend to spread over neighbouring basidia and prevent their spores from being liberated.

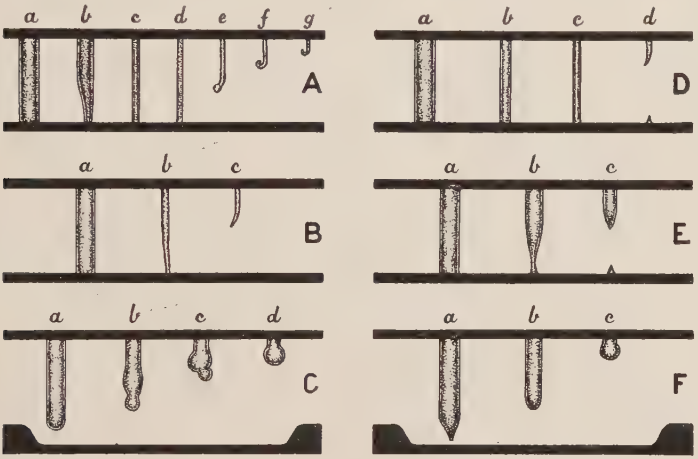


FIG. 123. *Coprinus atramentarius.* The autodigestion of six cystidia. *a*, in each drawing, is a fully turgid cystidium just before the beginning of its autodigestion. *b*, *c*, *d*, etc., show stages in the autodigestion of each cystidium. The cystidia were observed stretching across the interlamellar spaces near the autodigesting edges of the gills. A, B, C, and F, show cystidia which became withdrawn to one of the gills. D and E show cystidia which, after becoming more and more slender, snapped into two parts: one part became withdrawn to one gill, and the other to the other gill. C shows the autodigestion of a cystidium which has been separated, whilst still turgid, from one of the gills with which it was originally in contact by its rounded apical end. F shows the autodigestion of a cystidium which had been separated, whilst still turgid, from the gill in which it had originated. Its free pointed basal end is shown in *a*. Magnification, 80.

As if to diminish the chance of the cystidial fluid interfering with the spores after its liberation, there is a clear spore-free zone of hymenium immediately around each cystidium (Figs. 117 and 120, pp. 276 and 281).

The disappearance of the cystidia from the gills is so well timed that it seems likely that it is a regulated process. Whence comes the stimulus which acts upon the protoplasm of a cystidium and thus initiates its autodigestion? Possibly, when a basidium is discharging its spores, it sends out a stimulus in all directions which,

on arriving at the cystidia within a certain radius, causes the protoplasm within the cystidia to produce or liberate enzymes which attack the cell-walls. However, there is another possibility. The spores are developed and discharged from below upwards on each gill, and the order of their discharge appears to be dependent in part at least upon the order of their development ; and it may be that the order of autodigestion of the cystidia is merely dependent upon the order in which the cystidia develop. But hitherto the order of development of the cystidia has not been studied.

The Interlamellar Spaces.—The existence of the interlamellar spaces between the gills provides : (1) a space in which the basidia of opposing gill-surfaces can develop without touching one another and (2) sufficient room for the violent discharge of the spores in the region of spore-discharge. As I have pointed out in the first volume of these *Researches*, the spores of all Hymenomycetes, when moist, are very adhesive and, when brought into contact with one another, they stick together. If the spores on basidia of opposing gills were to touch one another during their development, it is probable that, during the subsequent expansion of the pileus, they would pull one another off their sterigmata, with the result that later on they would not be properly liberated. However, whilst the spores are ripening, opposing gill-surfaces are entirely prevented from coming into contact owing to the fact that they are kept apart by the numerous cystidia which act as distance-pieces.

The interlamellar spaces seem to be somewhat too wide, if we are to imagine that their one function is to provide space for the free development of the basidia and spores (*cf.* Fig. 122, p. 287). However, as my earlier studies of the mode of spore-discharge in the Agaricineae showed, the interlamellar spaces require to be sufficiently broad to permit of the spores being violently discharged into them without any risk of their striking and adhering to the opposing gills toward which they are propelled.[1] The spores of *Coprinus atramentarius* are shot forward from the basidia for an average distance of the order of 0·05 mm. It appears probable, therefore, that, with the provision of space sufficient for the liberation of the spores and with the further provision of a certain margin

[1] These *Researches*, vol. i, 1909, pp. 40, 211–212.

of safety which becomes of importance when the gills are more or less tilted (Fig. 124), the width of the interlamellar spaces has been reduced to a minimum. Since the width of the spaces is determined by the length of the cystidia, we may conclude that the length of the cystidia is correlated with the width of the space between the gills required to permit of the violent discharge of the spores in the region of spore-discharge.

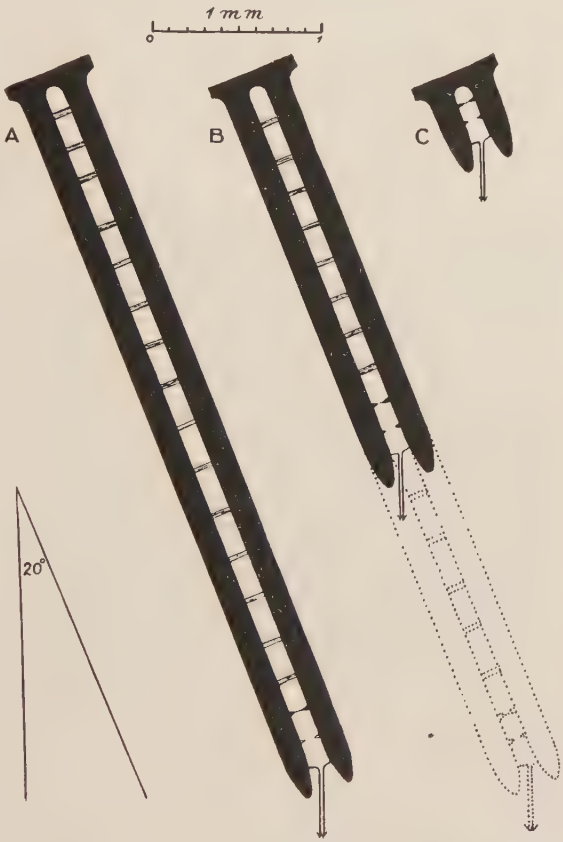


Fig. 124.—*Coprinus atramentarius.* Diagram illustrating the escape of spores from tilted gills. Two gills are tilted at an angle of 20°. Cystidia cross the interlamellar space and thus keep the gills apart. The trajectories of spores shot outwards from the zone of spore-discharge in still air are indicated by the arrows. A, soon after the beginning of spore-discharge. B and C show that, owing to autodigestion of those parts of the gills which have shed their spores, the upwardly-moving zone of spore-discharge is always just above the gill-edge. Magnification, 22.

The margin of safety in the width of the interlamellar spaces is drawn upon whenever the gills are inclined to the vertical when spore-discharge is proceeding. Since the gills are not positively geotropic, it usually happens that one side of a gill looks slightly upwards and the other slightly downwards during spore-discharge; but, even when the gills are tilted to an angle of 20° (Fig. 124), the spores can still escape without hindrance. This is provided for by the margin of safety in the width of the

interlamellar spaces and by suitable relations between the distance to which the spores are shot away from their basidia and the distance of the discharging basidia from the extreme edges of the autodigesting gills.

Summary concerning the Structure and Function of the Cystidia.—From the facts which have now been recorded it seems clear that the cystidia of *Coprinus atramentarius* are fitted for their distance-piece function by their shape, size, contents, position, mode of attachment at each end, number, and early development ; whilst the time, order, and mode of their destruction are arrangements which prevent their becoming obstacles to the escape of the spores from the fruit-body. Even if we were to search throughout the whole range of the vegetable kingdom it would be difficult to find any more beautiful and perfect example of cellular specialisation and of adaptation of structure to function than is here afforded us.

The Pilei of Coprinus atramentarius and C. comatus compared.—The replacement of the flange arrangement for separating the gills by the cystidial permits of a greater compactness of the gills in the fruit-body. Every one who has examined an expanding fruit-body of *Coprinus atramentarius* must have been struck by the thinness and closeness of the gills and by the unusual solidarity of the mass which they make up. On the other hand, an expanding fruit-body of *Coprinus comatus* or *C. sterquilinus* is not nearly so compact, as far as the gills are concerned. If one takes two fruit-bodies of equal diameter, one of *C. atramentarius* and the other of *C. comatus*, one finds by counting that there are about twice as many gills in the former as in the latter. This is due to the fact that the gills are thinner and the interlamellar spaces are narrower in *C. atramentarius* than they are in *C. comatus*. The cystidial arrangement for separating the gills, as developed in *Coprinus atramentarius*, evidently permits of a maximum number of gills being packed into the pileus. It also permits of the gills (which the cystidia support) being extremely thin and, at the same time, extremely broad. However, in *Coprinus comatus*, the flange arrangement of separating the gills, while involving the presence of fewer and narrower gills than the cystidial arrangement as exemplified in *C. atramentarius*, appears to allow of a partially compensating advantage, namely,

greater length of the gills. The gills of large fruit-bodies of *Coprinus comatus* are nearly twice as long as the gills of large specimens of *Coprinus atramentarius.*

After comparing the fruit-bodies of *Coprinus atramentarius* and *C. comatus*, I am inclined to regard the former as the more perfectly specialised, and this for two reasons : (1) the gills of *C. atramentarius* are thinner than those of *C. comatus* and therefore per unit of mass have a larger hymenial surface, and (2) the whole pileus of *C. atramentarius* is more compact than that of *C. comatus.* This superiority in compactness is due to the fact that the interlamellar spaces in *C. atramentarius* are reduced to a minimum and the gills are broad and short instead of being relatively narrow and long. The reduction of the width of the interlamellar spaces and the broadening of the gills are correlated with the presence of cystidia.

Fig. 125.—*Coprinus narcoticus* in pure culture on horse dung. The pilei are covered with a greyish-white floccose meal. Natural size.

The Pileus-flesh.—When one considers the structure of a large fruit-body of *Coprinus atramentarius*, *e.g.* that shown in section in Fig. 118, A (p. 278), the small amount of pileus-flesh relatively to the great size and number of the gills is very noticeable. A non-Coprinus fruit-body such as that of *Pluteus cervinus* (Fig. 47, p. 103), which possesses almost equally deep gills, has a very much greater amount of flesh. The amounts of flesh in the Coprinus and the non-Coprinus types are correlated with the different mechanisms employed for securing the successful production and liberation of the spores. The Coprinus mechanism is such that the flesh can be reduced to a minimum. This point, however, will be elaborated in more detail in the next Volume.

Other Coprini belonging to the Atramentarius Sub-type.—Among the Coprini belonging to the Atramentarius Sub-type are : *Coprinus narcoticus*, *C. urticaecola*, *C. stercorarius*, *C. macrorhizus*, and *C. picaceus*. In all these species cystidia cross the interlamellar spaces and interlock the gills during the discharge of the spores in the same manner as in *C. atramentarius*.

Coprinus narcoticus (Fig. 125, also Vol. II, Fig. 108, p. 318) has sometimes been confused with *C. niveus* (Fig. 126). It resembles

Fig. 126.—*Coprinus niveus* in pure culture on horse dung. The pilei are covered with a snow-white floccose meal. Natural size.

C. niveus in size, in having its pileus covered with a loose more or less squamulose meal composed of numerous, spherical, easily detachable cells, and in coming up on horse dung ; but it differs from *C. niveus* in its pileus being greyish-white instead of snow-white (*cf.* Figs. 125 and 126), in having trisporous basidia instead of quadrisporous (Vol. II, Fig. 109, p. 319), and in emitting a remarkably pungent odour during autodigestion instead of being practically scentless. Both *C. narcoticus* and *C. niveus* possess cystidia but, during spore-discharge, whereas those of *C. narcoticus* interlock the gills, those of *C. niveus* merely project from the gill-sides like pegs, as in *C. lagopus*.

Coprinus urticaecola was described and illustrated in Chapter VI (Figs. 54 and 55, pp. 133 and 136). Its pilei are small ; but the gills,

as in *C. atramentarius*, are very broad relatively to their length and are interlocked during the process of spore-discharge.

Coprinus stercorarius (Fig. 127) in size, colour, and general appearance very much resembles *C. narcoticus*, but the meal-cells on its pileus have a larger diameter, there are four spores on each basidium instead of three, the odour is faint instead of being remarkably pungent, and the fruit-bodies usually, although not always, spring from a small black sclerotium (Fig. 127). *C. stercorarius*, as its name implies, is found on dung. In my laboratory it frequently comes up on horse dung and cow dung. Experience has taught me that, on very wet dung, it succeeds better than most other Coprini. To obtain *C. stercorarius* at Winnipeg all that it is necessary to do is to obtain some fresh cow manure, put it in a large covered crystallising dish, and leave it on a laboratory table. Then, in the course of about two weeks, the fungus begins to produce its characteristic sclerotia at the surface of the dung. These sclerotia are at first white but soon turn black and, if left on the dung for a few weeks, germinate and produce fruit-bodies. In Fig. 127, on the left, is shown a fruit-body which is developing from a sclerotium while, on the right, are shown some fruit-bodies which have sprung from the hyphae of an ordinary mycelium.

FIG. 127.—*Coprinus stercorarius*. On the left, a fruit-body coming up on a sclerotium formed five weeks previously on wet cow dung. On the right, several fruit-bodies springing directly from an ordinary mycelium contained in cow dung. The left-hand pileus is covered with a greyish-white floccose meal. From the right-hand pileus the meal has been rubbed off. Natural size.

Coprinus stercorarius resembles *C. sterquilinus* and *C. narcoticus* in being homothallic and in thus differing from *C. lagopus*,

C. macrorhizus, *C. niveus*, and *C. Rostrupianus*, all of which are heterothallic.[1]

Coprinus macrorhizus (Fig. 128) often comes up on heating straw-manure, and then it develops a rooting base or pseudorhiza. It differs from *C. narcoticus*, *C. stercorarius*, and *C. niveus* in its pileus being covered with loose fibrous matted scales consisting of chains of elongated cells, instead of with loose squamulose meal consisting of spherical cells. It agrees with *C. lagopus* (Fig. 133, p. 305; also Vol. II, Fig. 20, p. 71) in having fibrous scales on the pileus, but differs from this species in having a stouter stipe and pileus, broader gills, smaller spores and, especially, as may be seen in the field, in its gills, during spore-discharge, being interlocked by cystidia instead of being free.

Fig. 128. *Coprinus macrorhizus*. Mature fruit-bodies shedding spores, growing on stable manure. The one on the extreme right has its pseudorhiza in view. Photographed at Birmingham, England. Natural size.

Coprinus picaceus (Fig. 129) is a beautiful species which I have seen growing in England at Taunton, Keswick, and near Birmingham.

[1] These sexual reactions were all determined in my laboratory; those of *C. macrorhizus* and *C. Rostrupianus* by Miss Dorothy Newton, the other five by Miss Irene Mounce. The problem of sex in the genus Coprinus will be fully treated of in vol. iv.

Mr. J. Ramsbottom has informed me that, during the Great War, he observed large numbers of the fruit-bodies coming up in a sandy

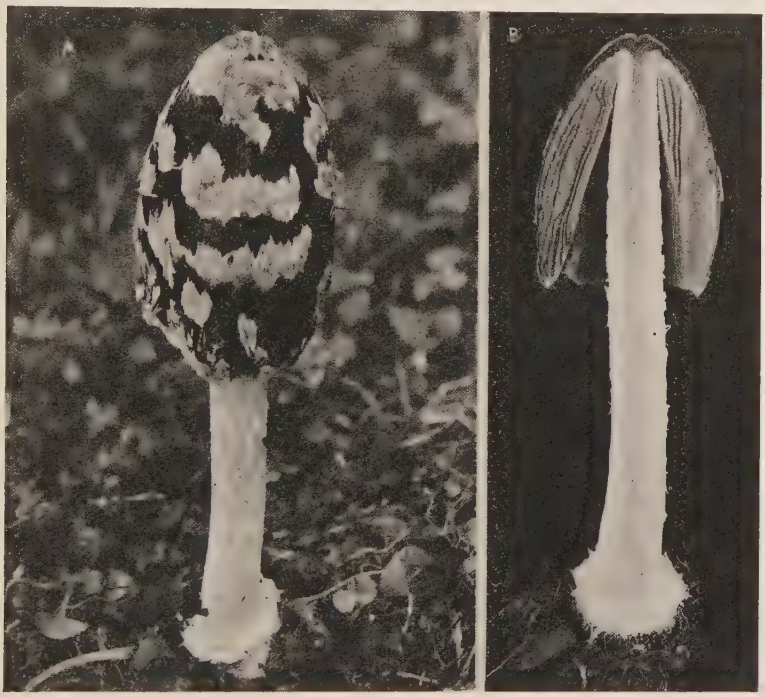


Fig. 129.—*Coprinus picaceus*, a member of the Atramentarius Sub-type, known by its dark ovate-campanulate pileus being variegated with broad white superficial scales. A, a fruit-body growing in the open. Its gills are about to liberate spores and undergo auto-digestion from below upwards. B, the same fruit-body in vertical section. Here, as in *C. atramentarius*, the gills are held tightly together by cystidia crossing the interlamellar spaces. Hence the gills in the plane of section have split down their median planes, thus exposing their tramae. The hymenial surfaces of the broken gills are to be seen as black longitudinal lines and the tramal surfaces as broad grey areas. Photographed at Keswick, England, in September, 1922, by Somerville Hastings and the author. Natural size.

place near Salonika. The species, while widely dispersed in Europe, appears to be absent from North America.[1]

[1] C. G. Lloyd (*Mycological Writings*, vol. v, Letter no. 68, p. 10) states that the North American Coprinus named by Peck a variety of *C. picaceus* does not resemble the *C. picaceus* of Europe. One of the late E. T. Harper's photographs of the Coprini, which have been deposited in the Field Museum at Chicago, is labelled *C. picaceus*, but I am sure this identification is erroneous: the fungus shown is not *C. picaceus*, but may be a scaly form of *C. atramentarius*.

Coprinus picaceus is readily distinguished from all the other large Coprini by having a pileus which is fuliginous-black variegated with broad, unequal, superficial, separating, white scales. The sharp contrast between the black pilear flesh and the large white scales is reminiscent of a similar contrast in the appearance of a magpie. Hence the specific name *picaceus*—pertaining to a magpie.

Coprinus picaceus has the largest cystidia I have yet observed in any Coprinus : they are about twice the size of those of *C. atramentarius* and even exceed in length those of *C. niveus*. In some fruit-bodies obtained at Taunton, which were half as large again as the one shown in Fig. 129, the cystidia could readily be seen with the naked eye interlocking the gills : they were 200–300 μ long and 50–60 μ wide.[1] In shape they were conico-cylindrical.

The interlocking of the gills by the numerous cystidia crossing the interlamellar spaces is so efficient in *C. picaceus* that, as in *C. atramentarius*, when one divides the pileus vertically downwards into two halves, the gills in the plane of cleavage split down their median planes, *i.e.* through the trama, each gill affected leaving one half of itself with one half of the pileus and the other half of itself with the other half of the pileus (*cf.* Fig. 129, B, with Fig. 111, p. 265). In the gill-region of Fig. 129, B, the black streaks alone are hymenial surfaces, while the broad grey areas are tramal surfaces.

[1] The cystidia of *C. picaceus*, like those of all other Coprini and of *Pluteus cervinus*, etc., grow in length apically for some time. My measurements were made on fully elongated cystidia at the time of spore-discharge. Ricken's measurements (*Die Blätterpilze*, Leipzig, 1915, p. 58), namely, 100-150 μ long and 30–50 μ wide, are considerably smaller than my own. The cause of this difference is at present uncertain ; possibly his cystidia had not yet grown to full length, or possibly the cystidia in his fruit-bodies were considerably smaller than in mine.

CHAPTER X

THE LAGOPUS SUB-TYPE ILLUSTRATED BY COPRINUS LAGOPUS

Characters of the Lagopus Sub-type—Representative Species—*Coprinus lagopus* and Allied Species—Description of *Coprinus lagopus*—Remarks on the Fruit-body Illustrations—The Scales on the Pileus—The Structure of the Gills—Autodigestion and the Liberation of the Spores

Characters of the Lagopus Sub-type.—The Lagopus Sub-type of fruit-body possesses all the essential characters already described for the Inaequi-hymeniiferous or Coprinus Type : (1) the gills are very thin, (2) the gills are parallel-sided, (3) the gills are not positively geotropic, (4) usually the hymenium on one side of a gill at maturity looks slightly downwards and that on the other side slightly upwards, (5) the spores ripen in succession from below upwards on each gill, (6) the spores are discharged in succession from below upwards on each gill, and (7) autodigestion proceeds from below upwards on each gill and removes those parts of the gills which have become spore-free and which, if they continued in existence, would become mechanical hindrances to the fall of the remaining spores.

The special characters of the Lagopus Sub-type which enable one to differentiate it from the other Coprinus Sub-types are as follows :

(1) The gills are parallel-sided and without conspicuous flanges. They therefore differ from those of the Comatus Sub-type which are subparallel-sided and flanged.

(2) Cystidia are present in considerable numbers on the face of the gills. In this we have a marked contrast with the Comatus Sub-type in which the hymenium consists of basidia and paraphyses only.

(3) The interlamellar spaces between the gills, which are required to render possible the free development of the spores on the hymenium, are secured by the cystidia which act as distance-pieces. In the young and unexpanded pileus the cystidia bridge the interlamellar spaces. This arrangement is similar to that of the Atramentarius Sub-type.

(4) The interlamellar spaces do *not* continue to be bridged by the cystidia during the discharge of the spores. Before the process of spore-discharge begins, the pileus expands umbrella-wise and adjacent gills become widely separated from one another, so that the cystidia no longer bridge the interlamellar spaces but merely project from the gill-sides like pegs. In this respect the Lagopus Sub-type forms a marked contrast with the Atramentarius Sub-type.

FIG. 130.—*Coprinus lagopus.* Fruit-bodies of medium size coming up spontaneously on horse-dung balls in the laboratory at Winnipeg. The stipes are elongating. The spores on the gills have become pigmented, hence the dark colour of the pilei. Natural size.

(5) The basidia are dimorphic, for they are of two lengths, long and short. In this character there is an agreement with most of the other Coprinus Sub-types but not with the Micaceus Sub-type where the basidia are tetramorphic.

(6) The pileus-flesh covering the gills is membranous. When the pileus expands just before spore-discharge, the flesh splits along radial lines corresponding with the lines of attachment of the gills, and each gill becomes split a certain distance down its median plane. Thus on the top of the pileus there comes into existence a series of radial sulcations which stretch from the pileus-rim to the disc. These radial sulcations do not arise in *Coprinus atramentarius* and certain other fungi of the Atramentarius Sub-type.

(7) The length of the gills relatively to the breadth tends to be greater than in the Atramentarius Sub-type.

The Lagopus Sub-type approaches very closely to the Micaceus Sub-type from which it differs in possessing dimorphic basidia instead of tetramorphic.

Representative Species.—The Lagopus Sub-type has been founded upon *Coprinus lagopus* but includes *Coprinus domesticus* (Fig. 25, p. 43), *C. echinosporus*, and certain other species, closely allied to *C. lagopus*, which hitherto have been confused with this species and have not been described in sufficient detail to permit of exact identification. In all these species: (1) the basidia are dimorphic and (2) the cystidia cross the interlamellar spaces only in the young unexpanded fruit-body and, as the pileus expands, become mere pegs projecting from the gill-sides.

FIG. 131.—*Coprinus lagopus*. Very small fruit-bodies coming up spontaneously on the under side of a horse-dung ball in the laboratory at Winnipeg. Natural size.

Within the Lagopus Sub-type may also be included the well-known *Coprinus niveus* (Fig. 126, p. 294), although it is somewhat aberrant in that its basidia vary from dimorphic to trimorphic.

Coprinus lagopus and Allied Species.—Unfortunately there is a good deal of confusion in the literature of mycology in respect to the nomenclature of *Coprinus lagopus* and its allies. It is therefore necessary to state that the fungus to which I attach this name is apparently the *C. lagopus* described by Lange[1] in his monograph of the genus Coprinus, and is certainly the *C. lagopus* carefully illustrated by Brefeld[2] in his *Untersuchungen*.

Coprinus macrorhizus (Pers.) Rea (= *C. fimetarius* Fr. var.

[1] J. E. Lange, "Studies in the Agarics of Denmark," *Dansk Botanisk Forening*, 1915, Part II, Coprinus, p. 41.

[2] O. Brefeld, *Untersuchungen über Pilze*, Heft III, 1877, Taf. VI, Fig. 1, *a—g*.

macrorhizus Pers.) is quite distinct from *C. lagopus*, for it differs from the latter in its well-developed pseudorhiza, its whiter and less fragile stipe, the more rounded and paler apex of its pileus, its broader and ventricose gills, its smaller and more oval spores, and particularly (a point which may be made out in the field with the naked eye or a pocket lens) in the fact that its cystidia, like those of *C. atramentarius*, bridge the interlamellar spaces and interlock the gills during the discharge of the spores (Fig. 128, p. 296). In both England and central Canada, on fresh horse-dung balls kept in covered crystallising dishes in the laboratory, *C. lagopus* almost always appears, but very rarely *C. macrorhizus*. *C. macrorhizus* most commonly occurs on warm straw-manure piles outside stables (Fig. 128). I have cultivated *C. lagopus* and *C. macrorhizus* on sterilised horse dung side by side in the laboratory and found that the two species are just as distinct from one another in cultures as they are under natural conditions. There can be no doubt that Rea [1] was fully justified in raising *C. macrorhizus* to specific rank. A good illustration of *C. macrorhizus* is to be found in Cooke's *Illustrations of British Fungi*, Plate 670; and a series of photographs and sketches of this fungus will be reproduced in Volume IV of this work.

The name *Coprinus fimetarius* seems to have been used in the past for two or more species resembling one another in having loose white fugacious flocci on the pileus and dense black spores. Sometimes the name has been applied to *C. lagopus* and sometimes to *C. macrorhizus*.[2] Since the specific name *fimetarius* has been used in such a confused manner, we are perhaps justified in following Rea's example and eliminating it altogether. Another name commonly found in the literature is *C. cinereus*. I am inclined to believe that *C. cinereus* has been used for a fungus which is identical with my *C. lagopus*.

From the above discussion it will be seen that *Coprinus macrorhizus* and *C. lagopus* are two distinct species, and that the names

[1] Carleton Rea, *British Basidiomycetae*, Cambridge, 1922, p. 503.

[2] The fungus illustrated by C. H. Kauffman in Plate XXXVIII of his *The Agaricaceae of Michigan* (Lansing, U.S.A., 1918) as *C. fimetarius* is certainly *C. macrorhizus*.

C. fimetarius and *C. cinereus* have been discarded on account of their being in all probability nothing but synonyms.

Description of Coprinus lagopus.—Since *C. lagopus* occurs so commonly in both Europe and Canada on fresh horse-dung balls confined in crystallising dishes in laboratory cultures, and since *C. lagopus* has been proved by Miss Mounce [1] to be heterothallic and may be used again for the investigation of sex problems, and since there has been, and still is, so much confusion in systematic works in respect to *C. lagopus* and its allies, I shall here give as careful a description of the species as I can with a view to making its identification less difficult to future workers :—

Fruit-bodies varying greatly in size from minute dwarfs with stipes 1–10 mm. high and expanded pilei 0·75–3 mm. in diameter occurring on old horse-dung balls in fields (Fig. 138, A and B, p. 316) to very large specimens with stipes 130–185 mm. high and expanded pilei 25–40 mm. in diameter occurring in pure cultures in the laboratory and sometimes under natural conditions in the open (Figs. 133 and 134 ; also Vol. II, Figs. 20 and 21, pp. 71 and 72). Fruit-bodies occurring spontaneously on horse-dung balls confined in large crystallising dishes in the laboratory (Fig. 130, p. 300) often with stipes 25–120 mm. high and expanded pilei 5–25 mm. in diameter. Dwarf fruit-bodies of this species were regarded by Massee as belonging to *Coprinus radiatus* ; but, since the mycelium produced from the spores of a dwarf give rise in pure cultures to very large fruit-bodies of *C. lagopus*, the separation of the dwarfs as a distinct species has no real justification.[2]

Pileus varying greatly in size, before expansion 0·5–20 mm. high (often about 6–10 mm. high), and after expansion 1–40 mm. wide (often 12–20 mm. wide) ; at first cylindric-oval and smooth under the down, then campanulate with a somewhat pointed apex and becoming radially grooved owing to the partial cleavage of each long gill down its median plane into two halves, then flattened, radially sulcate up to the small disc, and splitting into a few rays

[1] I. Mounce, " Homothallism and Heterothallism in the Genus Coprinus," *Trans. Brit. Myc. Soc.*, vol. vii, 1922, pp. 256–269.

[2] *Vide* " The Dwarf Fruit-bodies of *Coprinus lagopus*," these *Researches*, vol. ii, 1922, pp. 82–88.

at its periphery, and finally its rim becoming turned slightly

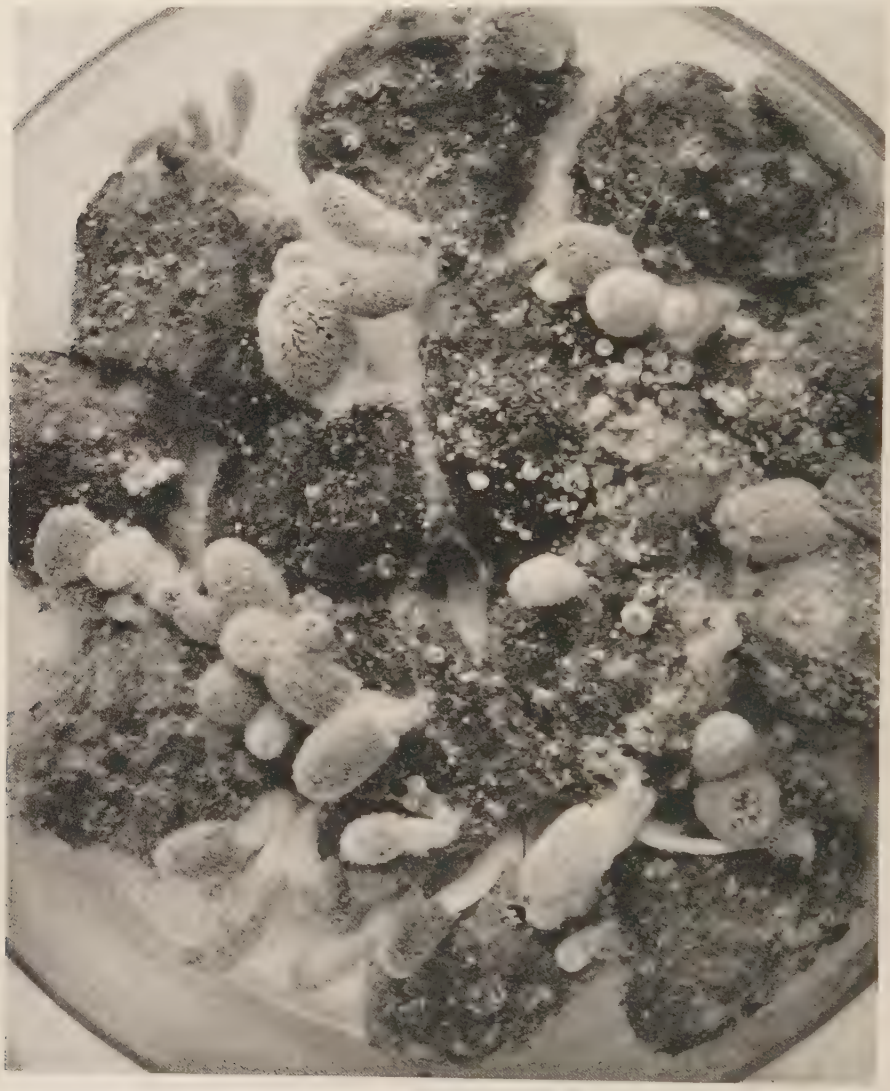


FIG. 132.—*Coprinus lagopus*. Pure culture from spores of English origin, on horse-dung balls at Winnipeg, 13 days after inoculation, showing numerous fruit-body rudiments on the surface of the dung and a crop of fruit-bodies which are about to elongate their stipes and expand their pilei. Each of these pilei is densely covered with soft hairy scales which are fragments of the veil (*cf.* Fig. 140, *v*). Natural size.

upwards without becoming converted into a roll; the disc, as the

pileus opens and flattens, ceasing to be prominent and becoming more or less depressed ; white whilst cylindric-oval and before the

FIG. 133.—*Coprinus lagopus.* To show variations in size of fruit-bodies grown from spores on horse dung in a pure culture. The pilei of the three fruit-bodies on the right are unexpanded, but those of the two on the left are expanding. The pileus of the central fruit-body is fully expanded, torn into rays, and revolute. The fugacious hairy scales on the pilei and the slender white fragile stipes are characteristic for this species. Natural size.

elongation of the stipe, owing to the presence of dense white down ; becoming grey during expansion owing to the presence of black spores on the enclosed gills ; and, finally, after the expansion of the pileus, black or blackish, owing to the separation of the white down into fugacious scales, the radial splitting of the gills, and the blackness of the spores showing through from below ; at first, before and

at the beginning of expansion, everywhere clothed with dense white down and, during expansion, the down becoming converted into small, thin, very delicate, easily detachable, fugacious, hairy tufts or scales consisting of matted chains of cells often 1 mm. or more long, a complete chain being composed of 8–10 more or less barrel-shaped cells, the smallest cells farthest from the pileus-flesh 60–100 × 20–30 μ and the largest next to the pileus-flesh 170–190 × 40–50 μ and in very vigorous fruit-bodies varying up to 240 × 80 μ.

Stipe, when fully extended, in dwarf fruit-bodies 1–10 mm. high, in large fruit-bodies varying up to 185 mm. (often 50–120 mm.); in large fruit-bodies the obconic base often swelling to 4–5 mm. in diameter, and occasionally to 7 mm., and showing a circular line where the pileus has broken away; shaft of stipe diminishing in diameter upwards, at the top in large fruit-bodies only 1–2 mm. in diameter and in smaller fruit-bodies much thinner; more or less clothed on the exterior with loose fibrillae, particularly on the lower half; watery white, hollow, very fragile; in small specimens rapidly withering when brought into dry air.

Gills at first white, at maturity black, adnexed, very thin, parallel-sided, autodigesting strongly at the edges, varying in size with the size of the pileus but length usually 6–10 times the breadth. The length and breadth of the gills of five fruit-bodies were: 6 × 1 mm., 10 × 1·3 mm., 15 × 1·5 mm., 23 × 2 mm., and 19 × 3·2 mm.

Pileus-flesh, in the unexpanded fruit-body white above gills and discoloured yellowish-brown at disc, after expansion appearing grey or black owing to the presence of black spores on the gills; before expansion, when the down has been rubbed off, very smooth, slippery, and shining; after expansion radially plicate to the disc owing to the cleavage of the gills, and often almost or quite naked owing to the fugacious scales having been removed by rain or other mechanical means.

Cystidia on the gill-sides (pleurocystidia) abundant in large fruit-bodies, very few in small ones; elongated-oval, rounded at the apex, somewhat bulging in the middle, and contracted into a stalk at the base; length 100–130 μ, breadth 35–45 μ; colourless; before the expansion of the pileus each cystidium completely

bridging an interlamellar space, with both ends firmly attached to the gills and held by clasping, often somewhat enlarged paraphyses; during the expansion of the pileus each cystidium breaking away at its apex from one gill and then projecting from the other gill like a peg.

Basidia dimorphic, quadrisporous; bodies of long basidia about 40 × 8–10 μ, of short basidia about 23 × 8–10 μ; sterigmata short.

Spores, very black in the mass on white paper, black and opaque when seen singly under the microscope; elongated-oval, round in transverse section, lower half slightly thicker than the upper half, about equally rounded at each end; 15–16·5 × 8–9 μ in large fruit-bodies, slightly smaller in dwarfs; smooth.

Common on horse-dung balls in the open, and frequently springing up spontaneously on horse-dung

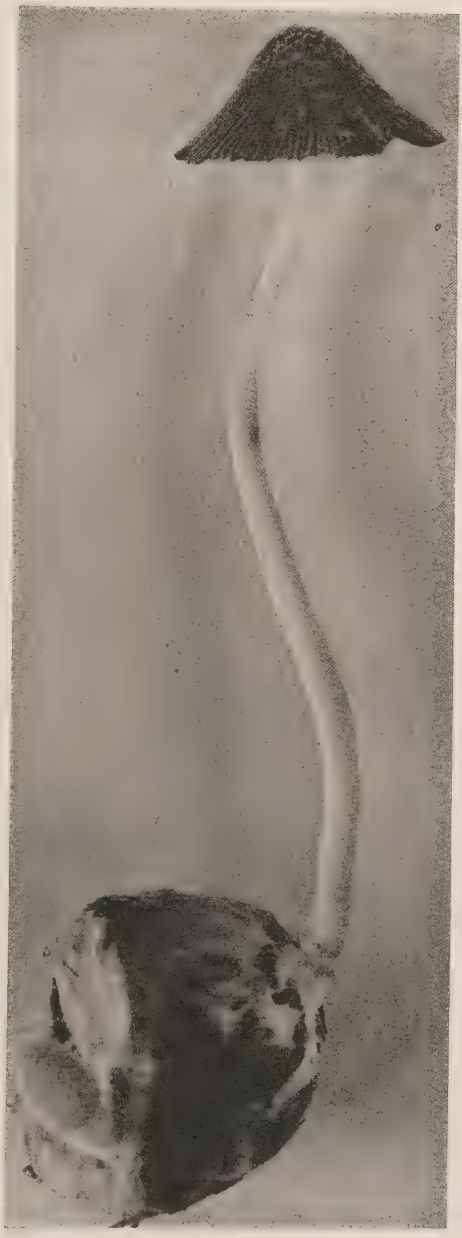


Fig. 134.—*Coprinus lagopus*. A very large fruit-body obtained by sowing spores from a very small fruit-body, which originated spontaneously at Winnipeg (*cf.* Fig. 131), on a sterilised horse-dung ball in the laboratory. The pileus, which is expanding and beginning, or about to begin, to shed spores, still bears many of the characteristic fugacious pilear scales. · Natural size.

balls kept moist in the laboratory. Sometimes occurring on germinating seeds of Mangold, Beet, and Sainfoin (Fig. 141, D, p. 320). Comparison by means of pure cultures showed that the form of *C. lagopus* occurring at Birmingham, England, is identical with the form occurring at Winnipeg, Canada.

Known by the prominent white down covering the unexpanded pileus and breaking up into thin fugacious tufts or scales, the narrow lanceolate gills autodigesting strongly at their edges, the large black cylindric-oval smooth spores, the cystidia not connecting the gills during spore-discharge, the tomentose stipe-base, the watery-white very fragile stipe-shaft bearing loose white fibrillae, the absence of a well-marked pseudorhiza, great variability in size, and by its frequent appearance on isolated horse-dung balls.

Distinguished: (1) from *Coprinus macrorhizus* by the absence of a well-marked pseudorhiza, the narrower gills and more pointed greyer apex of the pileus, the cystidia not bridging the interlamellar spaces during spore-discharge, and by frequently coming up on isolated dung-balls; (2) from *C. echinosporus* by having spores which are smooth instead of verrucose, rounded at the apex instead of truncate, and relatively much larger; (3) from a species which may be called *C. flavo-lanatus* by having white instead of yellowish-white down on the pileus, by the down-cells being unbranched and much thicker, and by having stouter cystidia; and (4) from another closely allied species which may be called *C. brevi-lanatus* by having longer and more patent down on the pileus just before expansion, and by the down-cells being much thicker.[1]

Remarks on the Fruit-body Illustrations.—As already indicated, the fruit-bodies of *Coprinus lagopus* vary greatly in size and general appearance. For this reason, and to facilitate identification in the future, the species is illustrated in this Chapter by a number of photographs and drawings. A few remarks will now be made concerning the fruit-bodies shown in the illustrations and the conditions under which they developed.

[1] All the four species mentioned in this paragraph have been grown in pure cultures in the laboratory and have been carefully studied with the microscope. *C. flavo-lanatus* and *C. brevi-lanatus* at present are *nomina nuda*, but I hope to describe them at some future time.

(1) *Wild fruit-bodies.* When fresh horse-dung balls collected from streets or stables in Winnipeg are kept for a few weeks in a large glass chamber in the laboratory, *Coprinus lagopus* fruit-bodies of medium size often make their appearance, as shown in Fig. 130 (p. 300) and at C and D in Fig. 138 (p. 316). Sometimes, however, on the under side of old dung-masses kept in the laboratory, smaller fruit-bodies come up, as shown in Fig. 131 (p. 301). Occasionally, on the under side of old horse-dung masses and in crevices between dung-balls, in fields in England and also on unsterilised horse-dung cultures in the laboratory at Winnipeg, one may see still smaller fruit-bodies—veritable dwarfs—with fully extended stipes 1–10 mm. long and fully expanded spore-bearing pilei 0·75–3 mm. wide. These extremely tiny fruit-bodies wither with the first breath of dry air and are too small to photograph satisfactorily. An account of them was given in Volume II,[1] and they are illustrated here by sketches reproduced at A and B in Fig. 138 (p. 316).

Under favourable conditions in the open, and sometimes on unsterilised horse-dung balls in the laboratory, fruit-bodies appear which are larger than those shown in Fig. 130 (p. 300) and more nearly resemble the cultivated fruit-bodies shown here in Fig. 133 (p. 305) and at E–N in Fig. 138, and in Volume II in Fig. 20 (p. 71).

There is no difficulty in obtaining wild fruit-bodies of *Coprinus lagopus* either at Winnipeg, Canada, or at Birmingham, England. All that one needs to do at either of these places is to take fresh horse-dung balls from stables and place them in a covered crystallising dish on the laboratory table. The dung-balls almost always contain *C. lagopus* spores which have been swallowed by the horse with grass-stems, etc.; and, at room temperatures, the first crop of fruit-bodies generally makes its appearance in from two to three weeks.

(2) *Cultivated fruit-bodies.* After the spores of a wild *Coprinus lagopus* fruit-body have been sown on fresh sterilised horse-dung balls in the laboratory, the mycelium which develops from the germ-tubes grows vigorously and produces fruit-bodies which are usually much larger than those which come up on unsterilised horse dung. Fruit-bodies, thus grown in pure cultures, are shown

[1] These *Researches*, vol. ii, 1922, pp. 82–88.

in Figs. 132, 133, and 134 (pp. 304, 305, and 307). Upon the dung in the crystallising dish of Fig. 132 a great many fruit-body rudiments made their appearance and a few of them, more favoured than the rest, have developed into young fruit-bodies. These have a soft woolly aspect, owing to the presence of white fibrillar scales upon their pilei; and their stipes, although at present very short, are about to elongate rapidly. In Fig. 133 the fruit-bodies are in various states of maturity. Their pilei are grey owing to the presence of black spores upon the gills, and the fibrillar scales are no longer so conspicuous. In Fig. 134 is to be seen the largest fruit-body which I have succeeded in raising under cultivation. Its pileus is rapidly expanding and is about to begin to liberate spores. The fugacious scales on its left side have been rubbed off by accident, thus exposing the gills which are splitting downwards radially from the pileus-periphery to the disc. There were loose white fibrillae on the surface of the stipe, but they are not visible in the photograph.

FIG. 135.—*Coprinus lagopus*. Fruit-bodies grown in complete darkness. Pure culture on a sterilised horse-dung ball, from spores originating at Birmingham, England. Note the woolliness of the stipes. The full-grown but as yet unexpanded pileus, owing to the unusual narrowness of the gills, is much thinner and more pointed than a pileus of equal height developed in the light (*cf.* Fig. 137). Its greyness shows that spores have ripened upon its gills and that it is therefore quite fertile. Natural size.

In Volume II, I pointed out that the tiny wild fruit-bodies occurring in crevices between, and under, horse-dung masses in fields in England, which Massee considered to belong to *Coprinus radiatus*, are in reality nothing but dwarf fruit-bodies of *C. lagopus*;

for, when their spores are sown on sterilised horse dung, the resulting mycelium gives rise to large fruit-bodies of *C. lagopus* resembling those of Fig. 133 (p. 305).[1]

(3) *Fruit-bodies grown in the dark.* Sterilised horse dung in two crystallising dishes was sown with spores of *Coprinus lagopus*; and, in the same room, one dish was placed on a table in the light, and the other dish in a cupboard from which all light was excluded. After from two to three weeks perfect spore-bearing fruit-bodies came up in both dishes. The fruit-bodies which had been exposed to daylight resembled those shown in Figs. 133, 134, and 138, E–N (pp. 305, 307, and 316), while those which came up in complete darkness are represented in Figs. 135, 136, and 138, O, P (pp. 310, 311, and 316). Both the lighted and the darkened fruit-bodies at first had white fibrillae on their stipes, and the woolliness of the stipes in Fig. 135 can be readily perceived. As compared with the lighted fruit-bodies, the darkened ones differed in: (1) having stipes which

FIG. 136.—*Coprinus lagopus*. Fruit-bodies grown in complete darkness. Pure culture on a horse-dung ball, from spores originating at Birmingham, England. Through having been handled, the pilei have lost their fugacious scales and the stipes their loose surface fibrillae. As in Fig. 135, the pilei are relatively thinner than pilei of equal height which have developed in the light (*cf.* Fig. 137). Their greyness shows that they have ripened spores upon their gills and are therefore fertile. Natural size.

[1] These *Researches*, vol. ii, 1922, pp. 86–87.

were relatively longer and which tapered toward the base (best seen in Fig. 136 where the white fibrillae have been removed), and in (2) having narrower gills and, relatively, a more pointed and narrower pileus (Figs. 135, 136, and 138, O, P). When one compares fruit-bodies grown in the light (Figs. 133, 134, 138, E–N) with fruit-bodies grown in complete darkness (Figs. 135, 136, and 138, O, P), it at once becomes evident that the form of the fruit-body of *C. lagopus* is moulded to some extent at least by the morphogenic stimulus of light. Here, as in *C. macrorhizus*,[1] light inhibits the development of the tapering pseudorhizal stipe-base, so that this only appears in fruit-bodies which have grown up in the dark.

(4) *Sterile fruit-bodies.* Sterile fruit-bodies of *Coprinus lagopus*, although but rarely or never seen under natural conditions, frequently occur in pure cultures of the type represented in Fig. 132 (p. 304). In Fig. 137 (p. 313), two sterile fruit-bodies are shown on the left and a fertile fruit-body of about equal size on the right. The sterile fruit-bodies are easily distinguished by their pale whitish-yellow colour and by the non-development of the spores, whereas the fertile fruit-body is blackish owing to the spores which are present in vast numbers on the gills. A similar comparison for some older fruit-bodies with expanding pilei is made possible in Volume II by means of Figs. 20 and 21 (pp. 71 and 72). In Volume II an illustration of the structure of a sterile gill is also provided (Fig. 22, p. 73).

There are degrees of sterility. Moreover, completely or partially sterile fruit-bodies may develop either in the light (Fig. 137) or in the dark (two pilei in centre of Fig. 136, p. 311).

Coprinus lagopus is heterothallic. Miss Mounce,[2] working in my laboratory with pure cultures, observed that the fruit-bodies arising from a secondary (diploid) mycelium produced by the union of two primary (haploid) mycelia of opposite sex are, as a rule, perfectly fertile and appear soon after the mating of the two primary mycelia has been effected, whereas the fruit-bodies arising from unmated primary (haploid) mycelia are sterile and develop tardily. This general association of fertility with the diploid condition of the mycelium and of sterility with the haploid condition, observed by

[1] *Vide infra*, vol. iv.

[2] These *Researches*, vol. ii, 1922, pp. 73–74.

Miss Mounce, has been abundantly confirmed by W. F. Hanna, another investigator working in my laboratory. Mr. Hanna made seventy new monosporous cultures of *C. lagopus*. Every one of the seventy mycelia produced fruit-bodies. In some cultures the fruit-bodies never elongated their stipes or expanded their pilei, in others the stipes elongated slightly and the pilei expanded slightly and, in yet others, the stipes elongated and the pilei expanded in a normal manner. In every one of the seventy cultures, however, the fruit-bodies were sterile in a greater or less degree. The gills were invariably white or whitish and never decidedly grey or black. In many fruit-bodies there was a complete absence of spores, while in other fruit-bodies a few spores could be detected by examining the gills with the microscope.

FIG. 137.—*Coprinus lagopus*. Fertility and sterility of fruit-bodies. Some large fruit-bodies grown from spores on sterilised horse-dung balls in the laboratory at Winnipeg. The one to the right, with the grey pileus, has developed spores normally (it shed them subsequently). The other two are colourless, showing that they have not developed their spores (they remained sterile and did not produce a spore-deposit). *Cf.* Figs. 20 and 21, Vol. II, pp. 71 and 72. Natural size.

In one fruit-body, raised by Mr. Hanna on a mycelium which originated from a single spore, although the gills were whitish, a considerable number of spores were produced, although far fewer than in normal fruit-bodies. These spores, however, were not discharged from their sterigmata and therefore did not give rise to any spore-deposit on a glass slide placed beneath the pileus. As autodigestion of the gills proceeded, black lines were formed at the gill-edges owing to the accumulation there of the undischarged spores. Mr. Hanna removed the spores from the gills and sowed

several hundreds of them together. Many of them germinated, but the compound mycelium resulting did not develop any clamp-connections, from which fact we may conclude that *the spores were all of one and the same sex.* Further experiment showed that the sex of these spores was the same as that of the single spore from which had arisen the primary mycelium which had given rise to the fruit-body. This observation confirms a similar one made by Hans Kniep [1] with *Schizophyllum commune.*

As pointed out in Volume II, when a great many spores of *Coprinus lagopus* are sown on sterilised horse dung in a large crystallising dish, sterile fruit-bodies often come up among the fertile ones. It was surmised that, in all probability, these fruit-bodies arise not on unmated primary mycelia but on secondary mycelia resulting from the union of primary mycelia of opposite sex.[2] Mr. Hanna has now proved this by experiment. With sterilised forceps he took a piece of the stipe of a sterile fruit-body which had come up on a culture of polysporous origin and placed it upon nutrient dung-agar. Hyphae at once began to grow from the stipe into the surrounding medium. When examined with the microscope, these hyphae were found to have developed clamp-connections, thus proving that the hyphae of the stipe, and therefore of the fruit-body as a whole, were of a secondary or diploid nature. Thus we have clear evidence that sterile fruit-bodies of *C. lagopus* are produced not only by primary mycelia but also by secondary mycelia.

In general terms, the cause of sterility in fruit-bodies of *Coprinus lagopus* appears to be lack of vigour in development. This lack of vigour in haploid fruit-bodies arising on haploid mycelia is doubtless due to their abnormal unisexual condition, while in diploid fruit-bodies arising on diploid mycelia in sterilised dung cultures the lack of vigour may be due to partial starvation brought about by the development of more fruit-bodies than the mycelium can properly support with nutriment.

The fact that monosporous mycelia of *Coprinus lagopus* give

[1] Hans Kniep, "Über morphologische und physiologische Geschlechtsdifferenzierung," *Verhandl. der Physikal.-med. Gesellschaft zu Würzburg*, 1919, p. 11.

[2] These *Researches*, vol. ii, 1922, pp. 73–74.

rise only to partially or completely sterile fruit-bodies enables us to infer with confidence that the normal fully fertile fruit-bodies of this species, which we see coming up on unsterilised horse dung in fields or in the laboratory, have all been produced on secondary mycelia, *i.e.* on mycelia which have resulted from the fusion of two mycelia of opposite sex.

(5) *Vertical sections through fruit-bodies.* In Fig. 138 is shown a series of drawings which illustrate not only how the fruit-bodies of *Coprinus lagopus* vary in size but also the appearance of the fruit-bodies in vertical section.

In Fig. 138, the fruit-bodies shown at A, B, C, and D were found upon unsterilised horse dung at Winnipeg, and they range in size from the tiniest dwarfs (A) to a fruit-body of medium size (D). All the other fruit-bodies, E–P, were raised in pure polysporous cultures on horse dung in the laboratory. E–N came up in the light, and O and P in total darkness, the latter exhibiting a strong tendency to form attenuated stipe-bases or pseudorhizae (*p*). Successive stages in development are shown in the series of drawings : K, L, E and F, M, N or O, G, H, I, J, P. The gills are at first white (K, L), then blacken from below upwards owing to the ripening of the spores from below upwards (M), and finally become black all over (N) ; so that the pileus, at the time the stipe begins to elongate, appears from without to be cinereous grey. After the stipe has elongated considerably, the pileus expands rapidly (G, H), flattens, and commences to shed spores (I), finally becoming more or less revolute and torn into rays (J). Autodigestion of the gills from below upwards causes the gills to become gradually reduced in area (J) until, finally, when spore-discharge has ceased, the remains of the gills and the pileus-disc are left at the top of the stipe as a watery remnant of the originally much larger pileus (P).

In Fig. 138 are also shown some variations in the relative width of the gills. The fruit-bodies M and N, raised in pure cultures on sterilised horse dung, were unusually vigorous and have extremely wide gills ; while the fruit-bodies G, H and I, raised in a similar manner, have normal narrower gills. The fruit-body O, also raised in pure culture on sterilised horse dung, but in total darkness

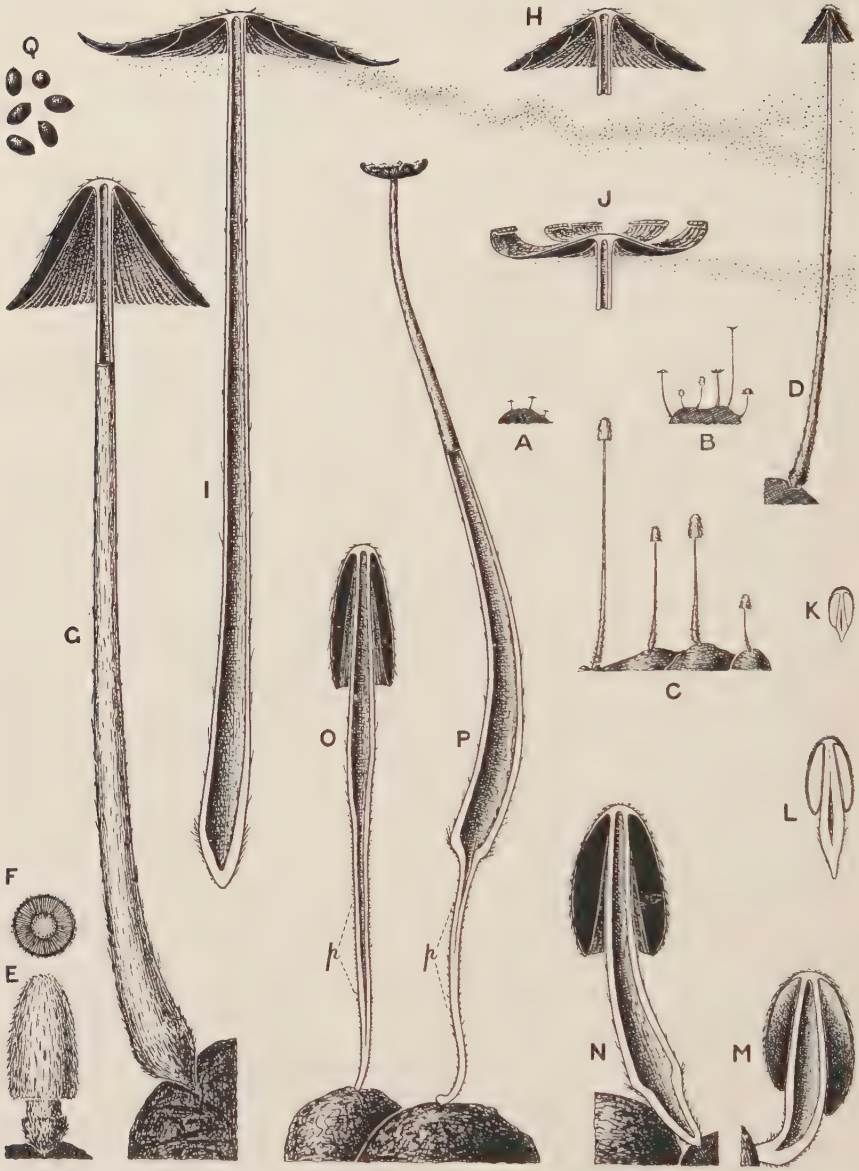


FIG. 138.—Fruit-bodies of *Coprinus lagopus*. Variations in size, and vertical sections. A, dwarf fruit-bodies on horse-dung, fully expanded, the smallest observed. B, dwarf fruit-bodies of slightly larger size, on dung. C, larger fruit-bodies, just before the expansion of the pileus. D, a still larger fruit-body (medium size). A–D, all observed on unsterilised horse dung at Winnipeg. E, a fruit-body in a pure culture on horse dung, young, the stipe about to elongate rapidly. F, a cross-section through the pileus of E, showing the stipe and radiating gills. G, H, I, J, four fruit-bodies grown from spores in pure cultures on horse dung: G, with the pileus rapidly expanding; H, with the pileus more expanded; I, with the pileus almost flattened and shedding spores from its gill-edges; J, with the pileus now revolute, the gills reduced by autodigestion from below upwards, and the spores still being discharged in large numbers. K, L, M, N, four fruit-bodies in pure cultures on horse dung, showing successive stages in development. In K and L the gills are white; in M the gills are blackening from below upwards owing to the gradual ripening of the spores; in N the gills are uniformly black. In M and N, which were grown in a large glass jar on sterilised horse-dung, the gills are exceptionally wide (*cf.* the gills of G and H). O and P, two fruit-bodies grown in pure culture on horse dung, not in the light, but in total darkness. Each has a slender stipe-base or pseudorhiza *p*. The gills of O are very narrow (*cf.* I and N). Q, spores, like those being discharged by I and J, but enlarged. Fruit-bodies all of English origin except A–D and J. All fruit-bodies shown natural size; magnification of the spores at Q, 293.

instead of in daylight, has, relatively to their length, the narrowest gills so far observed.

The stipe of *Coprinus lagopus* is extremely fragile. This is due to the fact that it is a hollow tapering cylinder with a weak and watery wall. The thinness of the stipe-wall relatively to the space enclosed may be readily perceived from an inspection of the fruit-bodies represented in Fig. 138 at I, M, N, O, and P.

The Scales on the Pileus.—One of the most noteworthy characters of the *Coprinus lagopus* fruit-body is the delicate scaliness of the pileus just before and during pilear expansion (Figs. 130,

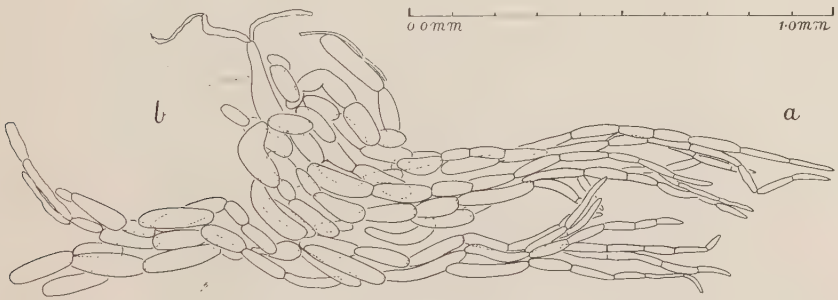


Fig. 139.—*Coprinus lagopus.* A single fugacious hairy scale removed from a large pileus beginning to expand, mounted in water, and sketched with a *camera lucida*: *a*, the contracted apical end directed away from the pileus; *b*, the broader basal end next to the pileus-flesh. The size can be measured by the scale.

132, and 133, pp. 300, 304, and 305). The scales are soft, white, hairy or fibrillose, more or less conical, in large fruit-bodies 1–1·5 mm. long, broadest where they are attached to the pileus-flesh, and having a free end projecting into the air (Figs. 139 and 141, B, *a*). In young unexpanded fruit-bodies (Fig. 132, p. 304) they give the pileus a very white and woolly appearance, but in older, expanded pilei they are not nearly so obvious (Figs. 133 and 134, pp. 305 and 307).

In a very small and rudimentary fruit-body of *Coprinus lagopus*, as soon as, with the help of microtome sections, one can distinguish the primordia of the gills, pileus-flesh, and stipe, it becomes evident that the universal veil covering the pileus is developing in such a way as to produce chains of cells directed away from the pileus-flesh and curved stipewards, each chain being in contact with other

chains. The result of this development is that a compact and radially constructed mass of cells comes to cover the pileus (*cf.* *v* in Fig. 140). As the pileus increases in size, this layer ceases to

Fig. 140.—*Coprinus macrorhizus.* Photomicrograph of a vertical section through a very young fruit-body showing: gills, *g*; pileus-flesh, *f*; the stipe, with its elongating pseudorhizal portion, *s*; and the veil, *v*. At this stage the veil is compact but, as the pileus grows larger, it breaks up into loose, fugacious, fibrous scales, as in *Coprinus lagopus*. Magnification, 32.

develop and is therefore forced to split radially between the cell-chains at more or less regular intervals, thus becoming broken up into a large number of small fragments (Fig. 132, p. 304). It is these fragments which form the scales. The scales of *Coprinus macrorhizus* and of some other species of Coprinus arise in an exactly similar manner.

If a scale is removed from a pileus which is about to expand and is examined with the microscope (Fig. 139), it is found to have the larger cells of the cell-chains next to the pileus-flesh and the smaller at the scale's free end. Whilst the pileus is increasing in size and thus causing the veil to split into scales, many of the cell-chains become more or less loosened or broken away from the pileus-flesh. For this reason, and also owing to the extreme delicacy of the cell-chains of which each scale is composed, the scales become very fugacious, the slightest rub removing them from the pileus, and exposure to dry air causing them to wither. Particularly during rain in the open, but also under laboratory conditions (Figs. 133, oldest fruit-body, 134, and 136, pp. 305, 307, and 311) the scales in older fruit-bodies tend to disappear completely; and sometimes on an expanded pileus no trace of them can be found.

The shape and size of the cells on the top of the pilei are important aids in distinguishing species of Coprinus from one another. On this account I shall here add a few more details regarding the cell-chains of *Coprinus lagopus*. The largest cells in a chain tend to be elongated-barrel-shaped or fusiform (Fig. 141), and their absolute size tends to vary directly as the size and vigour of the fruit-body which produces them, the largest fruit-bodies having the largest cells and the smallest fruit-bodies the smallest cells. The variation in size of the largest cells in the cell-chains is indicated pictorially in Fig. 141 by means of comparative *camera-lucida* sketches. The cells nos. 7, 8, and 9 in A were the largest cells in a cell-chain of a fruit-body of medium size, the cells in B the corresponding cells for a large fruit-body, and the cells in C and D the corresponding cells for two very small fruit-bodies. In two well-developed fruit-bodies, one belonging to *Coprinus lagopus* and the other to *C. macrorhizus*, it was found that the largest cells in the cell-chains of the former species were distinctly stouter than those of the latter species.

When the cells in a cell-chain of *Coprinus lagopus* are fully grown, they have very thin cell-walls enclosing a very thin lining layer of cytoplasm and a large central vacuole filled with clear and colourless cell-sap. Sometimes, however, in addition to lining the

cell-wall, the protoplasm is extended as a coarse foam, the foam

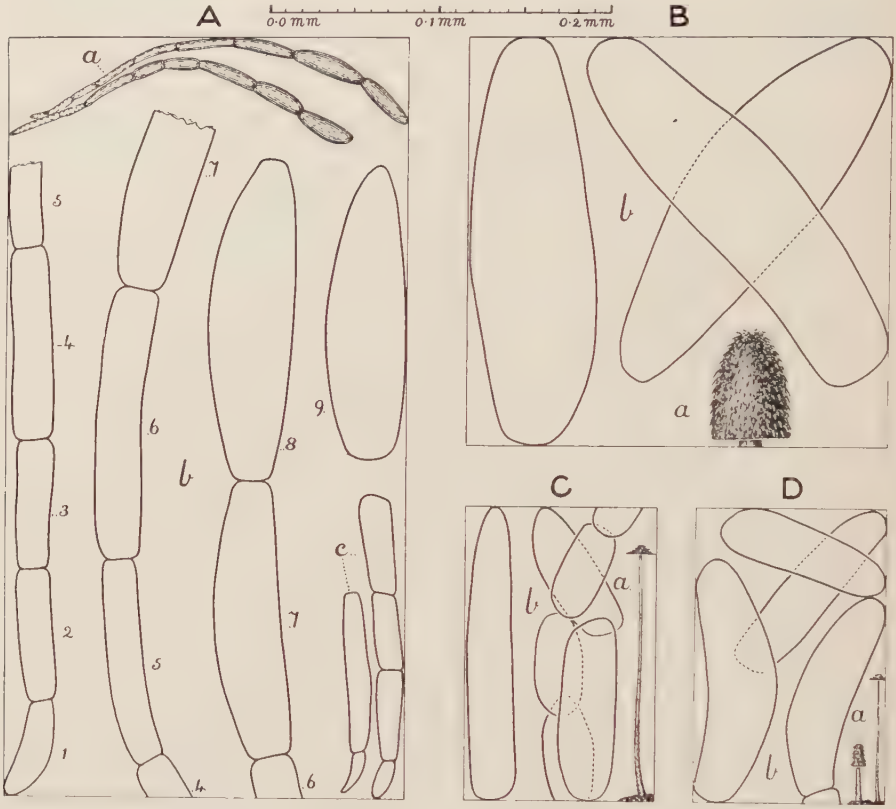


FIG. 141.—*Coprinus lagopus*. Size and form of the cells of the fugacious hairy scales on the pileus. A: from a *medium-sized* fruit-body in a pure culture; *a*, two cellular filaments of a single scale sketched with a magnification of 50 diameters, the larger cells were next to the pileus-flesh; *b*, the cells of the upper filament of *a* on a larger scale; *c*, the apical cells of two other filaments. B: *a*, *large* and vigorous fruit-body from a pure culture; *b*, three of the largest cells from filaments of scales on *a*. C: *a*, a *small* fruit-body coming up spontaneously on horse dung in the laboratory at Winnipeg; *b*, largest cells from the ends of filaments in its scales. D: *a*, two *very small* fruit-bodies which came up spontaneously on rotting seeds (mangold, beet, and sainfoin) in England; *b*, largest cells from the ends of the filaments in their scales. A comparison of A, B, C, and D shows that the largest cells in the pilear scales of *C. lagopus* tend to increase in size with increase in size of the fruit-body. The fruit-bodies all natural size. A, *a*, magnification 50. A, *b* and *c*; B, *b*; C, *b*; and D, *b*; magnification, 225; the size of each cell can be measured with the scale.

filling up one end of a cell or extending from a lateral wall toward the cell's central axis.

The firm and compact veil covering the top of a very rudimentary fruit-body (*cf.* Fig. 140) doubtless serves to protect the inner tissues of the pileus during their development. A fruit-body rudiment, as it grows in size, becomes negatively geotropic, and the elongation of the stipe then pushes the pileus upwards through crevices in dung-balls, etc. During its upward progress, the tip of the young pileus is shielded and assisted in sliding between obstacles by its veil in very much the same manner as, during its downward passage through the soil, the radicle of a seedling is

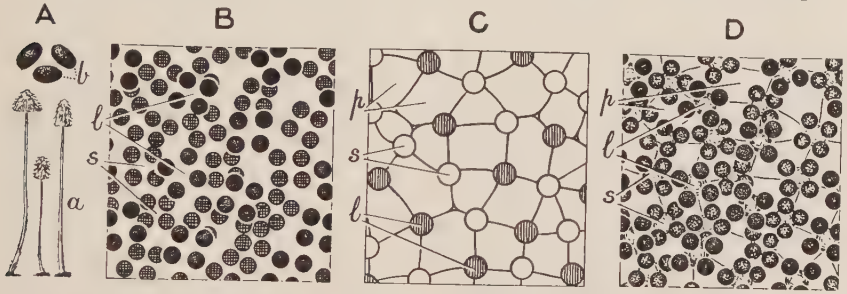


FIG. 142.—*Coprinus lagopus.* Study of a gill in surface view. Each of the squares in B, C, and D has a width of 0·01 mm. A : *a*, shows size of fruit-bodies used for the investigation ; *b*, some spores in side view. B, a *camera-lucida* drawing of the spores only : *l*, spores of long basidia ; *s*, spores of short basidia. C, a *camera-lucida* drawing showing a plan of : the long basidia, *l* ; the short basidia, *s* ; and the paraphyses, *p*. D, semi-diagrammatic drawing showing spores, basidia, and paraphyses in perspective : *l*, long basidia ; *s*, short basidia ; *p*, paraphyses. A, *a*, natural size. A, *b*, and B, C, and D, magnification, 293.

shielded and assisted in sliding between soil particles by its root-cap. The velar cap and the root-cap resemble one another both in their general form and in their slipperiness. When a fruit-body has increased somewhat in size and has emerged into the outer air, the velar cap is of no further use : as we have seen, it then ceases to grow and becomes broken up into delicate fugacious scales. While, therefore, the scales on the mature pileus of *Coprinus lagopus* appear to be quite functionless, we must ascribe to the veil, which gives rise to them, the function of protecting the pileus and assisting its movements when the fruit-body is in a very rudimentary condition.

The Structure of the Gills.—The gills of *Coprinus lagopus* are parallel-sided (Fig. 143) and are covered by a hymenium consisting

of basidia, paraphyses, and cystidia. The basidia, as in most Coprini, are dimorphic. The *camera-lucida* drawings B and C in Fig. 142, as well as the semi-diagrammatic Fig. 144, show : (1) that the basidia are packed closely together ; (2) that the spores of the long basidia often partly overstand the spores of the short basidia ; and (3) that the short basidia are more numerous than the long ones.[1] The paraphyses (Figs. 142, C, and 144) form a continuous pavement in which the basidia and cystidia are set at intervals. In Fig. 142, D, are shown in perspective the long and short basidia with their spores, and the paraphyses.

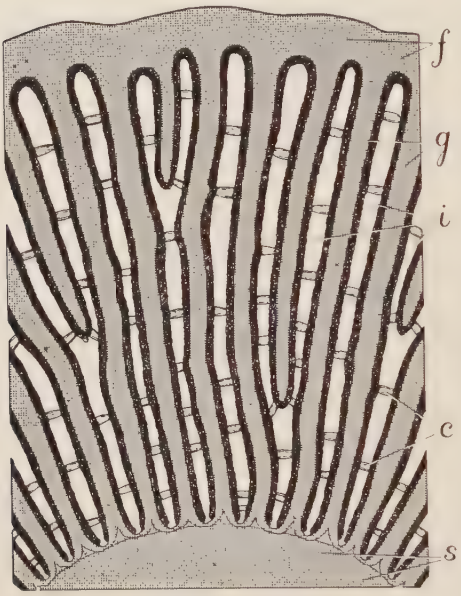


Fig. 143.—*Coprinus lagopus.* Semi-diagrammatic transverse section through the pileus and stipe of an unexpanded fruit-body : *f*, the pileus-flesh ; *g*, the gills ; *i*, the interlamellar spaces ; *c*, the cystidia ; and *s*, the stipe. The black layer at the surface of the gills is made up of the black spores of the long and short basidia and of the protuberant shafts of the long basidia. The illustration shows that the cystidia all bridge the interlamellar spaces and act as distance-pieces. Magnification, 29.

A transverse section through the gills of an unexpanded fruit-body is represented with low magnification in Fig. 143. Here one especially notices (1) the dark sides of the gills, the darkness being due to the projecting parts of the long basidia and to the two layers of spores, and (2) the cystidia crossing the interlamellar spaces. As shown in Figs. 144 and 145 in more detail, each cystidium is elongated-oval, rounded at the apex, somewhat bulging in the middle, and contracted into a stalk at the base. In an unexpanded fruit-body, both ends of a cystidium are firmly attached to the gills and are held by clasping somewhat enlarged paraphyses which, collectively, often form a bulge on the hymenium

[1] For a discussion of the packing of the elements of the hymenium in Coprini, *vide* pp. 154–155.

(*cl* in Figs. 144 and 145). If the gills are torn apart, one end of each cystidium remains attached to one of the gills, whilst the other becomes free. Very often the free end has attached to it some of

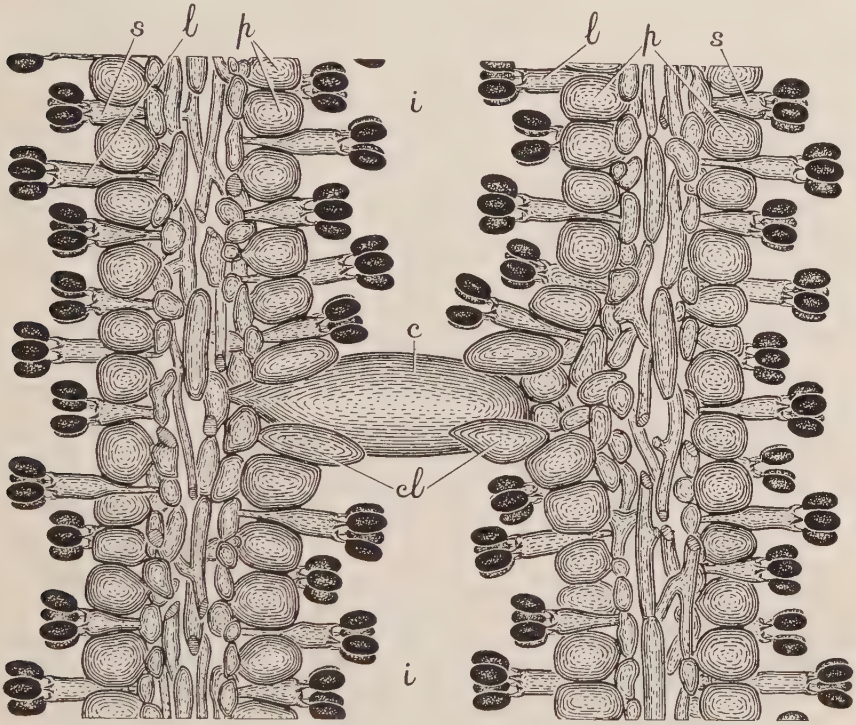


FIG. 144.—*Coprinus lagopus*. Transverse section through two gills of an unexpanded fruit-body (*cf.* Fig. 143), highly magnified. The hymenium consists of: short basidia, *s*; long basidia, *l*; and paraphyses, *p*. The subhymenium and trama are but small in amount and not sharply marked off from one another. The cystidium, *c*, crosses the interlamellar space, *i i*, and is attached to the two gills by both its ends. These ends strongly adhere to certain enlarged paraphyses or *clasping-cells*, *cl*. Magnification, 293.

the clasping cells, thus proving how strongly these cells adhere to the cystidial wall. In a young and unexpanded fruit-body, the cystidia, as in *Coprinus atramentarius*, act as distance pieces and keep opposing hymenial surfaces apart, thereby allowing the basidia to develop their sterigmata and spores in a free space.[1]

Autodigestion and the Liberation of the Spores.—The stipe of a fruit-body which is about to liberate spores elongates rapidly and,

[1] *Cf.* p. 275.

in the course of a few hours, raises the pileus from the surface of the substratum to its maximum height. Just as the stipe is ceasing to

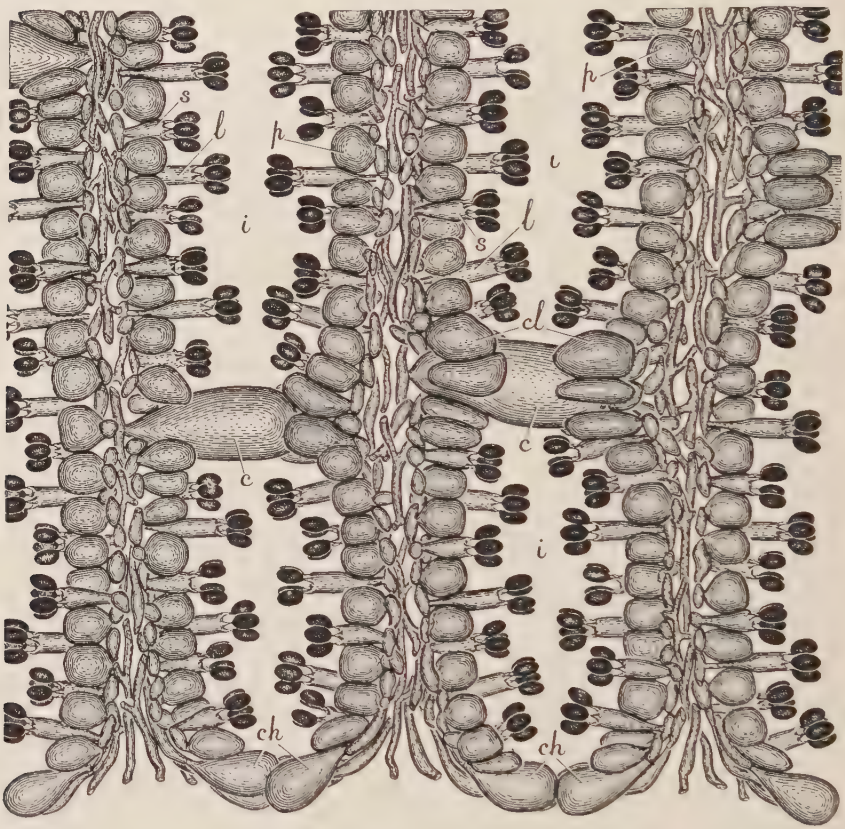


FIG. 145.—*Coprinus lagopus*. Semi-diagrammatic transverse section through three gills and two interlamellar spaces of an unexpanded fruit-body (*cf.* Fig. 143), to show how the gills are attached to one another by their edges close to the stipe : *ch*, cheilocystidia of adjacent gills attached to one another ; *c c*, cystidia (pleurocystidia) crossing the interlamellar spaces, *i i*, and attached to opposing gills by their ends ; *s*, short basidia ; *l*, long basidia ; *p*, paraphyses. Magnification, 225.

elongate, the pileus expands rapidly (Fig. 133, p. 305) and becomes more or less flattened. During the opening of the pileus, adjacent gills become widely separated and one end of each cystidium becomes loosened from the gill to which it was formerly so firmly attached, with the result that in an expanded pileus (Fig. 147, B) the cystidia no longer bridge the interlamellar spaces, but merely

project from the sides of the gills as pegs. If one examines with the microscope the under side of such a pileus as that shown at B

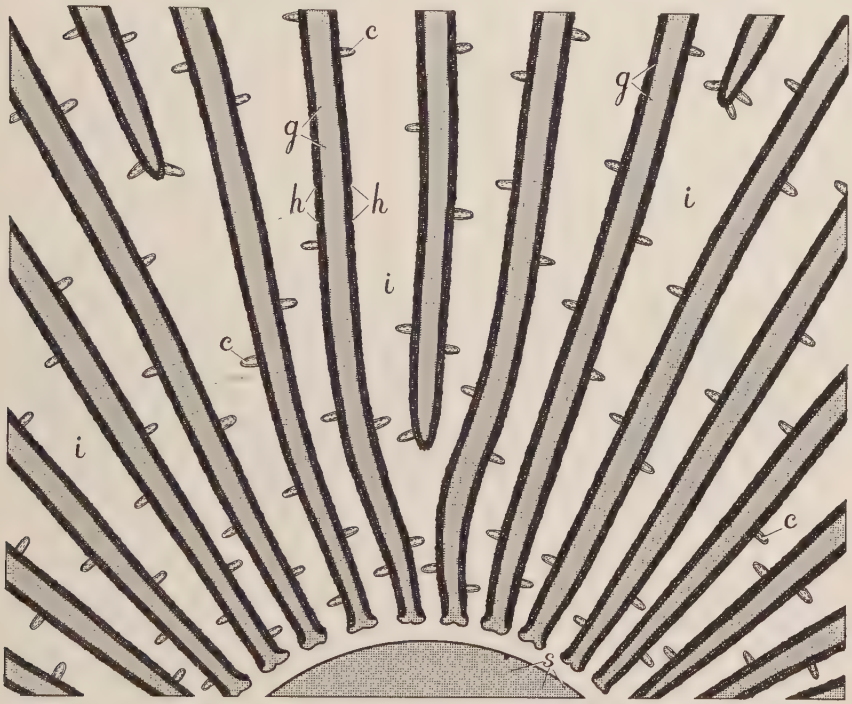


FIG. 146.—*Coprinus lagopus.* Semi-diagrammatic transverse section through the stipe, and longitudinal transverse section through the gills, of a fruit-body of which the pileus has become flattened owing to expansion. To show that the cystidia in an expanded pileus no longer cross the interlamellar spaces but merely project from the gill-sides like pegs. Spore-discharge is not visible, as it is taking place from the edges of the gills below the plane of section. An optical section like that here shown can be seen when an expanded pileus is turned upside down on a slide and viewed with the low power of the microscope. *s*, the stipe; *g*, *g*, gills; *h*, *h*, the dark layer made up of the protuberant parts of the long basidia and the black spores of the long and short basidia; *i*, *i*, interlamellar spaces; *c*, *c*, cystidia. Magnification, 29.

in Fig. 147, one finds that it has the appearance represented in Fig. 146.

In a young and unexpanded fruit-body, such as that shown in Fig. 138, M (p. 316), the edges of the gills are in contact with the stipe; and they are also united with one another by means of large cells which somewhat resemble the cystidia (pleurocystidia) on the gill-sides and which may be regarded as cheilocystidia. This union

of the gill-edges is illustrated in Fig. 145 at *ch*. When the pileus begins to expand, the cheilocystidia of adjacent gills separate from one another ; and, when autodigestion of the gills commences, the cheilocystidia are the first cells to be destroyed.

The discharge of the spores from below upwards on each gill and the autodigestion of the parts of the gills which have become spore-free take place as in *Coprinus atramentarius* (Fig. 138, H, I, J, P, p. 316). Here, too, the cystidia on each gill become autodigested in succession from below upwards in advance of the basidia and

Fig. 147.—*Coprinus lagopus*. A, a small fruit-body toward the end of its spore-fall period. The expanded pileus is ragged at the edge owing to autodigestion, but still bears some of its characteristic fugacious fibrous scales. B, an expanded pileus of considerable size, shortly after the beginning of the spore-fall period, seen from below ; shown to demonstrate that in *C. lagopus*, during the spore-fall period, adjacent gills are widely separated and not connected by bridging cystidia. The white peripheral zone is white owing to the loss of the black spores through spore-discharge which is proceeding centripetally. Winnipeg material. Natural size.

paraphyses by which they are immediately surrounded, so that the cystidia are prevented from becoming obstacles to the fall of spores shot outwards from the hymenium.[1] The reader who desires to visualise the microscopic appearance of a gill-edge of *Coprinus lagopus* during the discharge of the spores should examine Fig. 120 (p. 281) which, although drawn for *C. atramentarius*, would serve, with a few changes in the size of the elements, for *C. lagopus*.

At B in Fig. 147 is a photograph of the under side of a pileus shortly after the beginning of spore-discharge. Here the edge of the pileus is white, owing to the loss of the black spores which have been shot away from the basidia. Evidently, spore-discharge (indicated by the whitening of the hymenium) is progressing centripetally

[1] *Cf.* pp. 285–286.

from the pileus-periphery to the pileus-disc or, in other words, from what were originally the lowest parts of the gills to what were originally the highest parts of the gills.

During the discharge of the spores, the pileus of *Coprinus lagopus* gradually becomes more or less revolute, its edges turning up somewhat but never forming a neat roll such as one may often see in *C. comatus* and *C. niveus* (Fig. 138, J, p. 316). At the same time, when large, it often becomes torn into a few rays (middle fruit-body of Fig. 133, p. 305). The small fruit-body shown in Fig. 147, A, is far advanced in the process of spore-discharge, and autodigestion has caused the edge of its pileus to become ragged. In *C. lagopus*, autodigestion takes place strongly, so that the pileus rapidly melts down to a watery stump. Soon after spore-discharge has been completed, the stipe collapses and the whole fruit-body falls to the earth. Here, as usual in Hymenomycetes, the sporophore dies immediately after its period of usefulness as a spore-liberating agent has come to an end.

CHAPTER XI

THE MICACEUS SUB-TYPE ILLUSTRATED BY COPRINUS MICACEUS

Characters of the Micaceus Sub-type—*Coprinus micaceus*—Historical Remarks—Relations of *Coprinus micaceus* with its Substratum—General Description of the Fruit-bodies—The Name *Micaceus* and the Scales on the Pileus—Certain Fruit-bodies Compared—The Structure of the Gills—The Discharge of the Spores—Effect of the Weather on the Fruit-bodies

Characters of the Micaceus Sub-type.—The Micaceus Sub-type of fruit-body possesses all the essential characters already described for the Inaequi-hymeniiferous or Coprinus Type : (1) the gills are very thin, (2) the gills are subparallel-sided, (3) the gills are not positively geotropic, (4) usually the hymenium on one side of a gill at maturity looks slightly downwards and that on the other side slightly upwards, (5) the spores ripen in succession from below upwards on each gill, (6) the spores are discharged in succession from below upwards on each gill, and (7) autodigestion proceeds from below upwards on each gill and removes those parts of the gills which have become spore-free and which, if they continued in existence, would become mechanical hindrances to the fall of the remaining spores.

The special characteristics of the Micaceus Sub-type which enable one to differentiate it from the other Coprinus Sub-types are as follows :

(1) The gills are subparallel-sided and bear large cystidia at their margins.

(2) Cystidia are present on the faces of the gills and chiefly on those gill-halves which, before the expansion of the pileus, lie nearest to the stipe.

(3) The cystidia, in the unexpanded fruit-body, are not attached by both ends to opposing gills. Some of them stretch across the interlamellar spaces, but others do not and simply project from the gill-sides like so many pegs. The interlamellar spaces required to provide room for the free development of the basidia and spores are secured in part by the rigidity of the gills, in part by a suitable spacing of the gills where they adjoin the flesh and at their margins near the stipe, and in part by the cystidia which act as guards and prevent two adjacent gills from anywhere touching one another with their hymenial surfaces.

(4) The cystidia do not bridge the interlamellar spaces during spore-discharge. When spore-discharge is about to begin, the pileus expands, adjacent gills become widely separated from one another, and then all the cystidia project from the gills in a peg-like manner.

(5) The pileus-flesh covering the gills is membranous and provided with grooves which run radially above the longer gills. When the pileus is expanding, just before and during the early part of the spore-discharge period, these grooves open out. As expansion of the pileus proceeds, the grooves deepen in such a way that the longer gills become split down their median planes for a certain distance. Finally, the ends of some of the long gills at the pileus-periphery become completely split down their median planes into two halves with the result that, in the later stages of its expansion, the pileus becomes torn into rays.

(6) The basidia are *tetramorphic*. The longest set of basidia is very protuberant, the next longest a little less protuberant, the next longest still less protuberant, while the shortest set of basidia is not protuberant at all. In the zone of spore-discharge there are *four sub-zones of spore-discharge* corresponding to the four sets of basidia. In having tetramorphic basidia and four sub-zones of spore-discharge the Micaceus Sub-type is unique and shows the highest degree of hymenial complexity in the Coprinus Type.

Coprinus micaceus.—*Coprinus micaceus* (Fig. 148) is one of the commonest of the larger species of Coprinus. Both in Europe and North America its fruit-bodies, with their yellow ferruginous or date-brown rimosely split pilei, may often be seen coming up in

FIG. 148.—*Coprinus micaceus*. A group of fruit-bodies coming up among grass, just after the elongation of the stipe and the expansion of the pileus to the campanulate stage. Three of the pilei have had holes eaten in them by slugs. Photographed by A. E. Peck in Yorkshire, England. Natural size.

dense clusters about the stumps of Elms, Poplars, Beeches, and other trees, or above buried roots. Sometimes hundreds of fruit-bodies spring up simultaneously in one place and by their very numbers become so conspicuous that every passer-by, whether a botanist or not, is compelled to take notice of them and ask himself the question: how came they there?

Historical Remarks.—*Coprinus micaceus*, on account of its common occurrence, early attracted the attention of mycologists, and it was illustrated in a rough wood-cut by the famous botanist Clusius as early as 1601 in the very first monograph on fungi that was ever published.[1] It is now well known that *Coprinus micaceus*, like other Coprini, is edible; but Clusius thought that it was poisonous and placed it in the sixteenth genus of his *Fungi perniciales*.

The hymenium of *Coprinus micaceus* was examined by Link in 1809, but he entirely misunderstood its true nature.[2] What we now know as basidia were thought by him to be *thecae* resembling asci, and he stated that each theca contains four series of spores. His erroneous illustration, showing the basidia as asci, was copied into Nees von Esenbeck's textbook of fungi[3] in 1817 and was everywhere accepted as accurate. The powerful influence of suggestion was never more manifest; for, for a long time, with Link's illustration before them, mycologist after mycologist followed one another like sheep in discovering asci in the hymenium of Basidiomycetes. Among these misled mycologists were Ditmar, Nees von Esenbeck, Fries, Ehrenberg, Greville, Desmazières, Klotzsch, Krombholz, Corda, and Vittadini.[4] Thus it came about that for almost thirty years after the publication of Link's paper it was

[1] C. Clusius, *Rariorum plantarum historia. Fungorum in Pannoniis observatorum brevis historia*, Antwerpiae, 1601, p. 282. The *Brevis Historia* of Clusius was re-published in 1900 by G. de Istvanffi for a tercentenary celebration. In an appendix to the *Brevis Historia* Clusius illustrated and described a few fungi not supposed to have been included in the *Brevis Historia*; and among these, apparently, is *Coprinus micaceus* (base of p. 293).

[2] H. F. Link, "Nova plantarum genera e classe Lichenum, Algarum, Fungorum," *Schrader's neues Journ. f. d. Bot.*, iii, 1809, pp. 10–15.

[3] Nees von Esenbeck, *Das System der Pilze und Schwämme*, Würzburg, 1817, Tab. XXV.

[4] Illustrations of basidia represented as asci were published by all these authors.

universally believed that the Hymenomycetes produce spores in the same manner as the Ascomycetes. At length, after the microscope had been considerably improved, Link's error was detected; and, between 1836 and 1842, Ascherson, Léveillé, Berkeley, Corda, and Phoebus raised their voices in chorus announcing the discovery of the true nature of basidia including the formation of the spores on the ends of the sterigmata. The honour of giving the first comprehensive and accurate account of the structure of the hymenium of the Hymenomycetes rests with Léveillé[1] whose classical paper appeared in 1837; but, in the same year, Corda[2] independently published a correct description of the structure of the hymenium of *Coprinus petasiformis* in the first volume of his *Icones Fungorum*, and his text was accompanied by excellent illustrations of a gill in surface view and in cross-section, showing very clearly the basidia, paraphyses, and cystidia, of which the hymenium is made up. *Coprinus petasiformis*, judging from Corda's illustrations of the very protuberant basidia and of a clustered group of the fruit-bodies, seems to have been a form of *Coprinus micaceus*. In having been thus associated with Link's unfortunate error and Corda's success in the first attempts to elucidate the true structure of the hymenium of Hymenomycetes, *Coprinus micaceus* will always be of particular interest to the student of the history of mycology.

Relations of Coprinus micaceus with its Substratum.—The relations of *Coprinus micaceus* with its substratum still require to be worked out. To what extent the fungus is able to destroy wood is not at present known. Usually the fruit-bodies are found coming up on soil or turf or sometimes on sand. I have tried under these circumstances to find the mycelium passing from the base of the stipes to some woody substratum below, but, hitherto, my endeavours have not met with complete success. It seems probable that the mycelium, after having invaded its subterranean substratum, sends out hyphae or thin hyphal cords which grow upwards to the surface of the soil and there form the fruit-bodies which appear in

[1] J. H. Léveillé, "Recherches sur l'Hyménium des Champignons," *Ann. Sci. Nat.*, 2 sér., T. VIII, pp. 321–338.

[2] A. C. I. Corda, *Icones Fungorum hucusque cognitorum*, Pragae, Bd. I, p. 26, Tab. VII.

such large numbers, this series of events resembling that already described for *Psathyrella disseminata*.[1] Sometimes I have been able to find short ozonium-like strands of an orange colour at the very base of the stipe, but their presence does not appear to be constant nor could I trace them to their organic substratum.

A large Elm (*Ulmus campestris*) with a trunk about four feet in diameter was cut down near Birmingham, England. About five years afterwards I visited the spot and found that *Coprinus micaceus* was coming up about and upon the stump. A number of fruit-bodies were pushing their way out between the loosened bark and the wood. On removing the bark, I found that the fruit-bodies were arising not from the wood but from the bark, and that the mycelium was feeding upon the thick layer of secondary phloem. Pieces of the bark were taken home and put in a closed tin box. After some days it was found that the mycelium had grown out from the bark in the form of orange-yellow strands, some of which projected into the air.[2] These observations go to show that the mycelium of *Coprinus micaceus* is able to flourish upon secondary phloem. Whether or not it can attack secondary wood remains to be investigated. It is possible that some saprophytic Agaricineae are specialised for destroying wood-masses and others for destroying bark, and that *Coprinus micaceus* belongs to the latter group.

Coprinus micaceus has been successfully cultivated from spores in the laboratory by Levine.[3] This observer found that the spores germinate in tap-water after they have been immersed therein for about twenty-four hours. He therefore sowed spores in tap-water and, when they were germinating, transferred them to sterilised culture media. The following media were employed: (*a*) soil in which the plants had been growing, (*b*) soil mixed with horse dung, (*c*) horse dung, (*d*) pieces of wood taken from the same soil, (*e*) beef agar, (*f*) carrot, (*g*) beans, (*h*) beet leaves, (*i*) string beans, (*j*) horse-dung agar, and (*k*) corn-meal agar. Whenever the medium

[1] *Vide* Chap. ii, pp. 30–36.

[2] The orange-yellow strands of hyphae were reminiscent of the ozonium of *Coprinus domesticus*.

[3] Michael Levine, "The Origin and Development of the Lamellae of *Coprinus micaceus*," *American Journal of Botany*, vol. i, 1914, p. 347.

became too dry, sterilised water was added by means of a pipette. Forty to sixty days after the media had been inoculated with spores,

Fig. 149.—*Coprinus micaceus*. Fruit-bodies removed from soil near a dead Poplar tree. The six young fruit-bodies in a group are not yet shedding spores : scales are present on the pilei. On the left is one very large fruit-body and two medium-sized ones, all undergoing autodigestion and shedding spores. The fruit-body on the extreme right which is in an advanced stage of autodigestion is also shedding spores. Photographed at Birmingham, England. Natural size.

young fruit-bodies appeared on the soil, horse dung, and corn-meal agar. Levine, who merely wanted to obtain fruit-bodies in their

earliest stages of development so that he might study the origin of the gills, does not tell us what kind of organic matter the soil contained. So far as I know, *Coprinus micaceus* has never been found upon horse dung; and corn-meal agar is a laboratory product. Levine's experiments upon the culture of *Coprinus micaceus*, therefore, although interesting in themselves, do not throw much light upon the manner in which the fungus carries on its vegetative existence under natural conditions.

General Description of the Fruit-bodies.—Some fruit-bodies in various stages of development are shown in Figs. 149 and 150. They grew beneath a large dead Poplar tree (*Populus serotina*) and were photographed in the open immediately after they had been dug up. They were therefore unspoiled by handling and exhibit their natural forms.

The pileus at maturity is from about 3 to 6 cm. in diameter and the mature plants are from 8 to 11 cm. (3–4·3 inches) high.

The pileus is submembranaceous, elliptical, then campanulate, then under moist conditions helmet-shaped, the edges becoming revolute and torn into rays (Fig. 150). When young, the pileus is ochraceous-tan in colour, the disc being darker, and covered with delicate scales composed of globular cells which glisten somewhat in the light (Fig. 151, p. 339). The scales tend to occur in radial lines (*cf.* the youngest fruit-bodies in Fig. 149) and are so loosely attached that they are removed from the pileus by the slightest touch or by a shower of rain. Older pilei are therefore often quite naked. The unexpanded pileus exhibits prominent striations which pass radially from the pileus-periphery to the disc. These striations mark the positions of furrows in the flesh and are situated above the lines of attachment of the longer gills (*cf.* Figs. 149, p. 334, and 152, p. 341). As the pileus expands, the furrows open out and the top of the pileus becomes radially rimoso-sulcate (Fig. 150). As it grows older and spore-discharge takes place, the pileus turns to a darker hue.

The gills are 2–3 cm. long and 0·4–0·8 cm. broad, somewhat rigid owing to their being about 0·3 mm. thick in their halves nearest to the pileus-flesh, becoming lanceolate during autodigestion so that in the middle stage of spore-discharge they are sharply

FIG. 150.—*Coprinus micaceus*. A group of fruit-bodies removed from soil near a dead Poplar tree. The fruit-bodies, which are still shedding a few spores, are in an advanced stage of autodigestion. The gills have disappeared from the underside of the revolute pileus-rims. Natural size.

pointed toward the pileus-periphery and broad toward the stipe, at first white, then from below upwards becoming brownish and finally blackish. Their free margins are covered with large cystidia. As the pileus expands, the gills become well separated from one another, and then the large cystidia present upon their sides can be seen as tiny pellucid pegs projecting freely into the interlamellar spaces (*cf.* Fig. 146, p. 325). Spore-discharge and autodigestion begin at the base of the gills before the upper parts of the gills have become completely blackened (Fig. 158, C and D, p. 354).

The stipe near its base is 0·4–0·8 cm. thick and tapers slightly as it passes upwards, so that at its top it is often only 0·2–0·4 cm. thick (Fig. 149, p. 334). It continues to lengthen as the pileus expands and whilst spore-discharge and autodigestion are taking place, so that in the end it may become 8–11 cm. long. It is hollow (Fig. 158, p. 354), brittle and whitish.

The pleurocystidia at maturity are 100–150 μ long, 40–65 μ wide, cylindrical or elongated-oval, equally rounded at both ends, stalkless or with an extremely short stalk, and easily detached from the gills which bear them.

The globular cells which make up the scales on the top of the pileus are colourless and have smooth walls from which crystals of calcium oxalate are absent (Fig. 151, p. 339). The diameter of the largest cells is about 65 μ, that of the smallest cells about 25 μ, and that of most of the cells from 40 to 50 μ.

The spores have three differing dimensions: length, breadth, and thickness. From above, they appear to be slightly oval (Fig. 155, A, p. 346) and, when seen in side view upon the sterigmata (Fig. 156, p. 348) or isolated in water, their diameters vary according to the way they happen to be turned to the observer. The spores are 8–11 μ long, 6–6·5 μ broad, and 5 μ thick. In water, a spore is pointed at its basal end and somewhat flattened at its apical end, while its maximum breadth is attained at a distance of about two-fifths of its length from the basal end. Individual spores, when seen in water under the microscope, are not deep black but brown. Spore-deposits on white paper are blackish-brown.

The fruit-bodies are densely caespitose and come up in woods, hedgerows, etc., about stumps and buried roots. In England they

may be seen from January to December.[1] Those shown in Figs. 149 and 150 were photographed on June 18. At King's Heath I found a cluster of fruit-bodies on December 22. In Manitoba I have met with fruit-bodies in the summer and as late as October 18.

Owing to variations in size, crowding, and habitat, owing also to changes during development and to the effects of the weather, the fruit-bodies of *Coprinus micaceus* are somewhat variable in appearance; and isolated fruit-bodies may sometimes be difficult to determine. They stand nearest to those of *Coprinus domesticus*, from which they are distinguished by their basidia being tetramorphic instead of dimorphic and by not possessing a well-developed red ozonium. *Coprinus congregatus* Bulliard, as illustrated in Cooke's *Illustrations of British Fungi* (Plate 679), seems to me to be simply a small form of *Coprinus micaceus*.

The Name Micaceus and the Scales on the Pileus.—The specific name *micaceus* was given to *Coprinus micaceus* on account of the fact that the large globular cells of the pilear scales and the large cystidia at the edges and on the faces of the developing gills glitter in the light like particles of mica.[2]

The very young pileus is mealy, *i.e.* is covered with a layer of globular cells of rather large size which can easily be removed by pressure from the finger. As the pileus grows in size, the mealy layer becomes broken up into mealy scales (Fig. 149, p. 334). The cells forming a scale, like those of *C. domesticus* but unlike those of *Coprinus niveus*, *C. narcoticus*, and *C. stercorarius*, do not very readily separate from one another but tend to cling together (Fig. 151, B). A few isolated scale-cells resting upon the palisade cells which bound the pileus-flesh on its exterior are shown in Fig. 151 at A.

The pileus of *Coprinus micaceus* was described by Fries[3] as "granulis micaceis fugacibus consperso," by Berkeley[4] as "sprinkled with glistening meal"; and these authors did not enter into

[1] Carleton Rea, "British Basidiomycetae," Cambridge, 1922, p. 506.

[2] P. Phoebus, "Ueber den Keimkörnerapparat der Agaricineen und Helvellaceen," *Nov. Act. Ac. Leop.-Carol. Nat. Cur.*, XIX, Pars II, 1842, p. 199.

[3] E. Fries, *Hymenomycetes Europaei*, Upsala, 1874, p. 325.

[4] M. J. Berkeley, *Outlines of British Fungology*, London, 1860, p. 179.

microscopic details. However, Massee[1] in his *British Fungus-Flora* states that "The pileus is covered with glistening minute crystals of oxalate of lime." I have examined a considerable number of pilei of *Coprinus micaceus* but have never seen any crystals of oxalate of lime upon them. It is true that minute crystals of oxalate of lime are present in the walls of the meal-cells of *Coprinus*

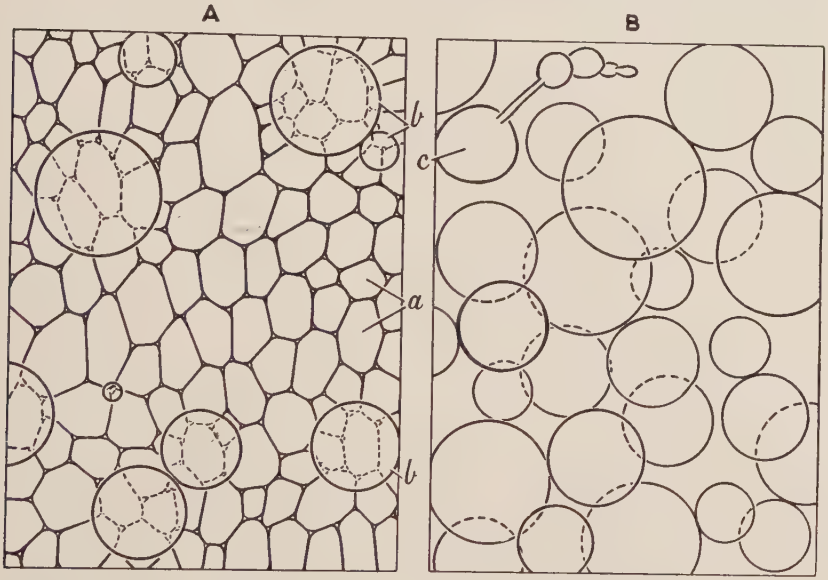


FIG. 151.—*Coprinus micaceus*. A, a surface view of a pileus showing: the outermost layer made up of palisade cells, *a*, and a few of the loose scale cells, *b*. B, some of the spherical cells making up the loose scales on the top of the pileus: their walls are free from crystals of calcium oxalate; *c*, a chain of cells showing mode of origin one from the other. Magnification, 293.

stercorarius and *C. narcoticus*, but this is not so with *C. micaceus*. In this species, as in *Coprinus niveus*, the walls of the meal-cells are quite smooth (Fig. 151). Occasionally I have found grains of sand upon the top of pilei of *Coprinus micaceus*, but it was evident that these mineral bodies had been carried up from the ground. Since an examination of the glistening particles making up the meal of *C. micaceus* reveals that they are composed of smooth-walled cells, there can be no doubt that Massee was in error in stating that the shining particles on the pileus consist of crystals of oxalate of

[1] G. Massee, *British Fungus-Flora*, vol. i, 1892, p. 317.

lime. The sparkling of the meal-cells, as well as of the cystidia on the edges and faces of the gills, is simply due to light which strikes them from without and is refracted and reflected to the eye in the same manner as from the minute drops of water one so often sees at the tips of grass leaves on English lawns early in the morning after a dewy night.

Certain Fruit-bodies Compared.—The chief differences between the fruit-bodies of *Coprinus micaceus* and those of the species already described, from the point of view of function, are associated with the structure of the gills and the mode of opening of the pilei.

The gills of *Coprinus micaceus* resemble those of *C. comatus* and *C. sterquilinus* and differ from those of *C. atramentarius* in that they are relatively thick and rigid and are also subparallel-sided.

The gills of *Coprinus micaceus* differ from those of *C. comatus* and *C. sterquilinus* and resemble those of *C. atramentarius* in that they do not possess conspicuous marginal flanges.

The gills of *Coprinus micaceus* resemble those of *C. atramentarius* and differ from those of *C. comatus* and *C. sterquilinus* in that they possess large cystidia on their sides. However, the cystidia of *C. micaceus* differ from those of *C. atramentarius* in that they are less numerous, are chiefly confined to the halves of the gills farthest from the pileus-flesh, are attached only to the gills from which they have originated, and act as distance-pieces only during the development of the basidia and spores and not during spore-discharge.

The pileus of *Coprinus micaceus* resembles that of *C. comatus* and *C. sterquilinus* and differs from that of *C. atramentarius* in that, before the process of spore-discharge begins, it opens out sufficiently to effect a wide separation of adjacent gills. In *Coprinus atramentarius*, throughout the spore-fall period, adjacent gills are connected laterally by the interlocking cystidia ; but in *Coprinus micaceus*, before any spores are liberated, adjacent gills become so far removed from one another that the cystidia can no longer stretch more than a fraction of the way across the interlamellar spaces. In having its interlamellar spaces unbridged by cystidia during the discharge of the spores, *C. micaceus* resembles *C. comatus* and *C. sterquilinus* and differs from *C. atramentarius*.

The gills of *Coprinus micaceus* differ from those of *C. comatus*,

C. sterquilinus, and *C. atramentarius*, as well as from those of the other species of Coprinus, in that the basidia are tetramorphic instead of being dimorphic. Four generations of basidia develop instead of two. In *Coprinus micaceus*, the zone of spore-discharge is subdivided into four subzones instead of the usual two.

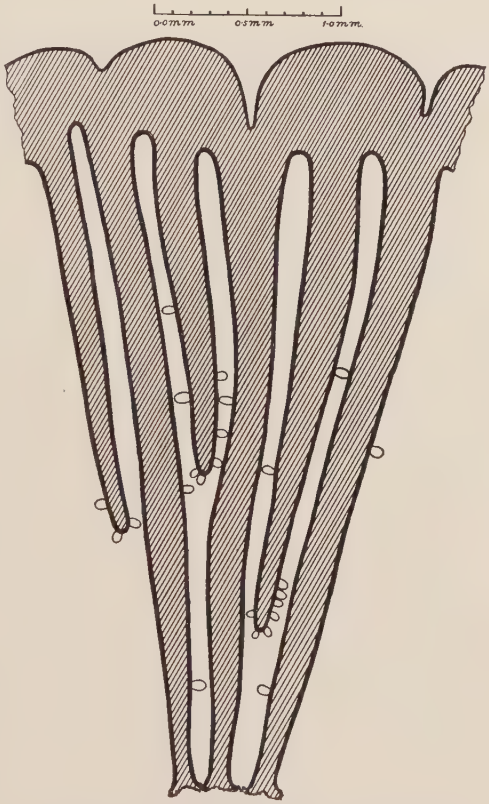


Fig. 152.—*Coprinus micaceus*. Shape and thickness of the gills. Horizontal section through part of an unexpanded pileus. Oval cystidia are present on the sides of the lamellae but, as a rule, do not stretch right across the interlamellar spaces. At this stage, basidia are developing but as yet are without spores. Magnification, 24.

The Structure of the Gills.—A transverse section taken through a pileus prior to the formation of the spores on the basidia and prior to expansion presents the appearance shown in Fig. 152. The furrows in the flesh, which open out as the pileus expands, correspond in position with the median planes of the long gills. The scale at the top of the Figure enables one to measure the thickness of the pileus-flesh and of the gills, and thus to realise how very thin they are relatively to those of the Mushroom and other aequihymeniiferous fungi. The short gills are more or less wedge-shaped and are packed neatly between the long gills. The long gills are for the most part subparallel-sided, but become contracted in thickness near the stipe ; and, just where they abut upon the stipe, they appear to be slightly swollen owing to their margins being clothed by large cystidia. Although the long gills are contracted

toward the stipe, in cross section they are far from having the regular and pronounced wedge shape so characteristic of the Aequi-hymeniiferae (*cf.* Fig. 152 with Figs. 85 and 139 in Vol. II, pp. 249 and 390).

The cystidia, which can be seen with the naked eye as glistening particles, are practically confined to the halves of the gills which are nearest to the stipe, and they are especially numerous at the free margins of the shorter gills (Fig. 152). In the young and unexpanded pileus some of them appear to stretch right across the interlamellar spaces, but others do not (Fig. 152). They certainly make it impossible for the opposing faces of adjacent gills to come into contact. After cutting and examining a number of sections like that shown in Fig. 152 as carefully as possible, I came to the conclusion that the distance-piece function of many of the cystidia is never called into play and, therefore, that many of the cystidia act merely as guards. The mechanical employment of any cystidium appears to depend on circumstances. During the development of the pileus, should two adjacent gills approach too near one another, the cystidia in the interlamellar space act as mechanical obstacles and prevent contact of the hymenial surfaces being made; but, should two adjacent gills not approach within a certain distance of one another, the cystidia project freely into the interlamellar space in a peg-like manner and have no mechanical work to do. This is a very different arrangement from that which has been already described for *Coprinus atramentarius*, where every cystidium without exception acts as a distance-piece until its destruction by autodigestion (*cf.* Fig. 122, p. 287).

On examining the hymenium of *Coprinus micaceus* in face view with the high power of the microscope, one can readily perceive the dark spores standing out upon a lighter background. An exact plan of the position of the spores over a two-hundredth part of a square mm. is shown at A in Fig. 153. At first glance, the spores on such an area may appear to be very irregularly arranged; but, if one focusses up and down with the aid of the fine adjustment, one quickly discovers that some of the spores stand in part vertically above others; so that, after all, there is no jostling of one spore by another. With a little care, one can resolve the spores into four sets according to the height at which they stand above

the general level of the hymenium, *i.e.* above the paraphyses. The four sets of spores which are really present in Fig. 153 at A, are shown differentiated from one another by shading in the same Figure at B. Everywhere in each set the spores occur in quartettes, and each quartette corresponds to a subjacent basidium. The four sets of spores, as developmental studies show, belong to four generations of basidia. The basidia of the first generation have the longest and most protuberant bodies ; the basidia of the second

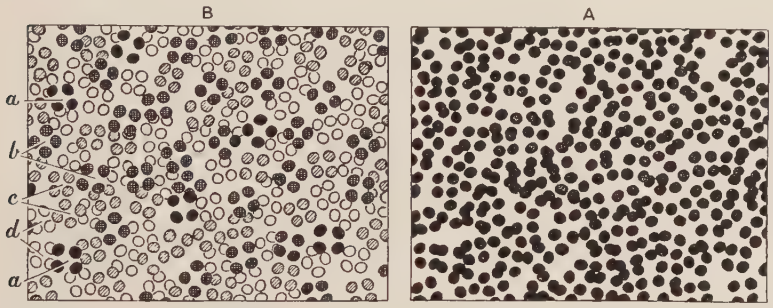


Fig. 153.—*Coprinus micaceus*. *Camera-lucida* sketches showing positions of the spores on the surface of a small area of the hymenium. A, the spores all represented as being in the same plane, to show crowding and overlapping. B, the same set of spores analysed according to generations ; *a*, (shown black), *b*, (cross-hatched), c, (shaded with lines), and *d*, (unshaded) are the spores of the basidia of the first, second, third, and fourth generations respectively. Spores of an older-generation basidium often stand partly over those of younger-generation basidia. Magnification, 293.

generation have somewhat shorter and less protuberant bodies ; the basidia of the third generation have still shorter and still less protuberant bodies ; while the basidia of the fourth generation have bodies which are the shortest of all and practically non-protuberant. The differential protuberancy of the four generations of basidia will be at once realised by a glance at Fig. 156 (p. 348), which represents the hymenium in cross-section. It is clear that the basidia are tetramorphic and that the bodies of the basidia of the first generation are a little more than twice as long as those of the basidia of the fourth generation. As shown in Fig. 156, the spores do not vary in size according to the length of the basidia upon which they arise ; for those on the shortest basidia are just as large as those on the longest basidia.

That the four sets of basidia which are present on the hymenium do really represent four generations was proved by studying the development of the hymenium upon a living gill seen in face view. At one stage of development of one small area of the hymenium, it was found (1) that the spores on the longest basidia were dark brown and therefore fully pigmented, (2) that the spores on the next longest basidia were brownish, (3) that the spores on the next longest basidia were just beginning to become coloured, and (4) that the spores on the shortest basidia were quite colourless. The longest set of basidia, the next longest set, the next longest set still, and the shortest set begin to develop their spores at successive intervals of time; and they likewise complete the development of their spores at successive intervals of time. It is clear that the development of the hymenium of *Coprinus micaceus* is similar to that of *Coprinus sterquilinus*, already so fully described, with the difference that in the former species there are four generations of basidia to be considered whereas in the latter there are only two. In *Coprinus micaceus* the wave of spore-development which passes upwards on each gill is compound; for, in reality, it is composed of four simpler waves, one for each of the four basidial generations.

The spores on any small area of the hymenium, when about to be discharged, are all equally dark brown in colour, as is indicated in Fig. 156 (p. 348). However, for the sake of diagrammatic clearness, in Fig. 153, B, the spores of the first, second, third, and fourth generations of basidia, respectively, have been uniformly blackened, shaded with crossed lines, shaded with simple parallel lines, and left unshaded. The relative numbers and positions of the basidia of each of the four generations present in Fig. 153, A and B, are made clear by the further analysis embodied in Fig. 154. Here at A, B, C, and D are shown the spores of the first, second, third, and fourth basidial generations respectively. Counting parts of spores at the edges of the drawings as wholes, the number of spores of the first, second, third, and fourth generations are 25, 112, 133, and 187 respectively. It thus appears that, proceeding from the first to the last generation on any given area, there is a progressive increase in density of distribution of the basidia.

The tetramorphism of the basidia of *Coprinus micaceus* has the

same general significance as the dimorphism of the basidia of *Coprinus comatus*, *C. sterquilinus*, and *C. atramentarius*. The spores on any small area of the hymenium, such as that represented in Fig. 153, A and B (p. 343), must all be ripe and ready for discharge at approximately one and the same time; for the zone of spore-discharge passes upwards across it and leaves it bare of spores in

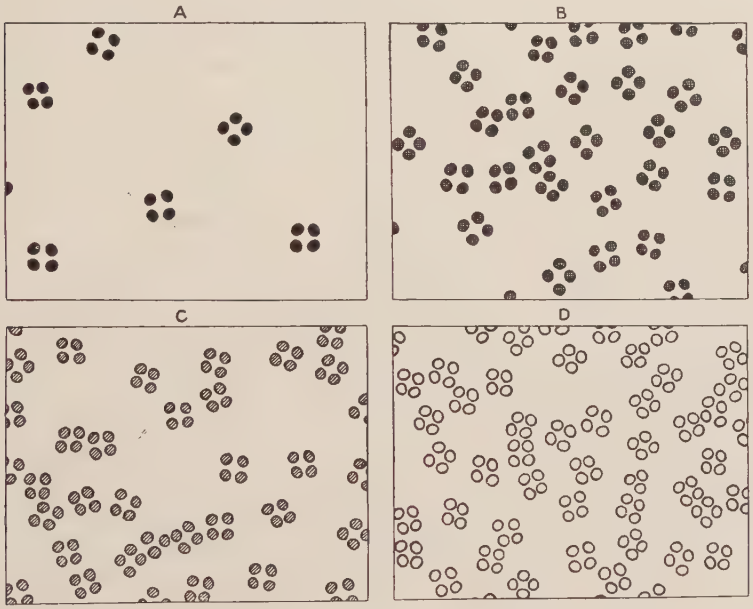


FIG. 154.—*Coprinus micaceus*. Analysis of the hymenium. The four generations of spores shown at B in Fig. 153 have been set out separately. A, B, C, and D show the spores of the basidia of the first, second, third, and fourth generations respectively. The shading of the spores is exactly the same as in Fig. 153, B. Magnification, 293.

the course of not very many minutes, while thereafter it is soon destroyed by autodigestion. Under these conditions more basidia, and therefore more spores, can be produced on any small area of the hymenium when the basidia are tetramorphic than when they are monomorphic. If all the spores shown in Fig. 153 at A had been produced upon equally high monomorphic basidia, during the production and discharge of the spores there would have been much jostling and disturbance. But the spores in question were produced on tetramorphic basidia, each set of basidia bearing its spores at a

different height above the paraphyses. As a consequence all jostling and mechanical disturbance was prevented and the hymenium produced and discharged its spores with the maximum of efficiency. *Coprinus micaceus* is the only species of Coprinus which, so far as I know, is so complex as to possess tetramorphic basidia ; but, as we have seen, tetramorphic basidia are present in the hymenium

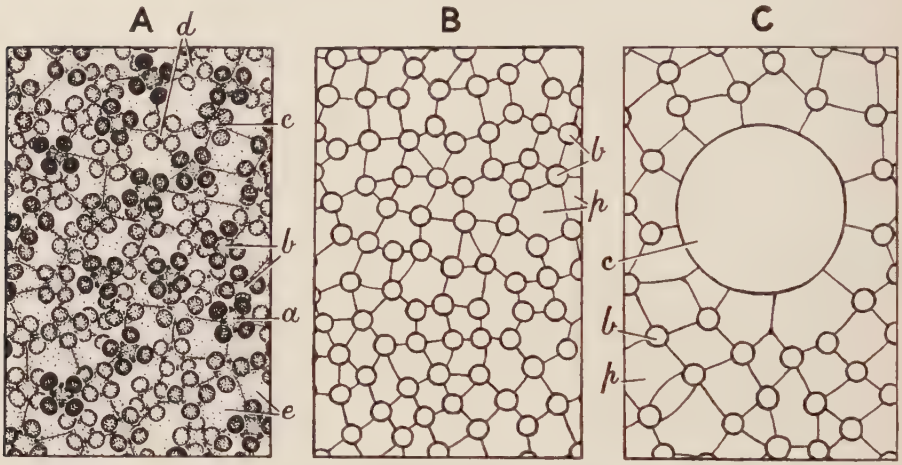


Fig. 155.—*Coprinus micaceus.* The hymenium in surface view. A, spores drawn with a *camera-lucida*, basidia and paraphyses added semi-diagrammatically, so as to show all the elements of the hymenium in one figure ; *a*, *b*, *c*, and *d* are basidia of the first, second, third, and fourth generations respectively ; *e*, paraphyses. B, *camera-lucida* plan of a piece of hymenium showing basidia, *b*, and paraphyses, *p*. C, a similar area but including a cystidium, *c*. Magnification, 440.

of certain non-Coprinus Agaricineae, namely, *Lepiota cepaestipes* and *Psathyrella disseminata* (Figs. 9 and 29, pp. 16 and 51).

The relative positions of the basidia and paraphyses are shown in Fig. 155 at B. Each basidium is surrounded and isolated from its neighbours by three or four or, more rarely, by five paraphyses. Here, as elsewhere in the Coprini, the sterile paraphyses are absolutely essential constituents of the hymenium in that they function as space-making agents : by their presence they prevent adjacent basidia, which simultaneously bear full-sized spores, from jostling one another during spore-development and spore-discharge. At the same time, they support the basidia and act as the elastic elements of the hymenium. The paraphyses are at first very small ;

but, as the pileus enlarges and opens out, they grow in size, thus enabling the hymenium to increase in surface area and so keep pace with the growth of the subhymenium and trama.

Another piece of the hymenium which happened to include a cystidium is shown in Fig. 155 at C. The cystidium has a diameter which is enormous relatively to the diameters of the basidia, and it is surrounded by paraphyses. For every cystidium there are thousands of basidia and paraphyses, so that, in comparison, cystidia are rare elements in the hymenium.

In Fig. 155 at A is shown a piece of the hymenium complete with basidia, paraphyses, and spores. The spores were drawn with the *camera lucida* from living material, so that their positions are true to nature. The basidia and paraphyses were added semi-diagrammatically. Optical difficulties prevent one from making a *camera-lucida* drawing of any one hymenial area that is complete in all its details. The spores must be surrounded by air when they are drawn, as the addition of water to the hymenium usually results in many of them being dislodged from their sterigmata ; but, in air, it is difficult to see the outlines of the paraphyses on account of the presence of the spores above them. Since water removes most of the ripe or almost ripe spores from their sterigmata, when one desires to make drawings of the basidia and paraphyses it is advisable to mount the gill in water. Other investigators do not seem to have attempted to draw complete and satisfactory surface views of the hymenium of Coprini ; but, as we have seen, the difficulties in the way of accomplishing this are not insuperable.

Occasionally, trisporous basidia occur among the quadrisporous, just as in the hymenium of *Coprinus sterquilinus*, *C. niveus* (Vol. II, Fig. 110 *b*, p. 320), etc. ; and two are present in the hymenium represented in Fig. 154, one in C and one in D (p. 345).

The cystidia vary from 100 to 150 μ in length and from 40 to 65 μ in width and, relatively to the basidia and paraphyses, are elements of gigantic size. In shape they are either elongated-oval, or they are cylindrical in the middle and bluntly rounded at each end like straight sausages. They are almost sessile on the sub-hymenial cells from which they arise, and it is only with the greatest difficulty that one can sometimes find a trace of a very minute stalk.

In Fig. 157, stalkless cystidia are represented at C, G, and H, and stalked cystidia at A, B, and F. When one cuts sections through the gills, some of the cystidia are often separated from the other cells of the hymenium and may then be seen lying isolated in the mounting fluid. The occurrence of such isolated cystidia in artificial preparations of *Coprinus micaceus* and certain related species was probably the fact which gave rise to the view held by Worthington Smith [1] and others that the cystidia of the Coprini drop out of the

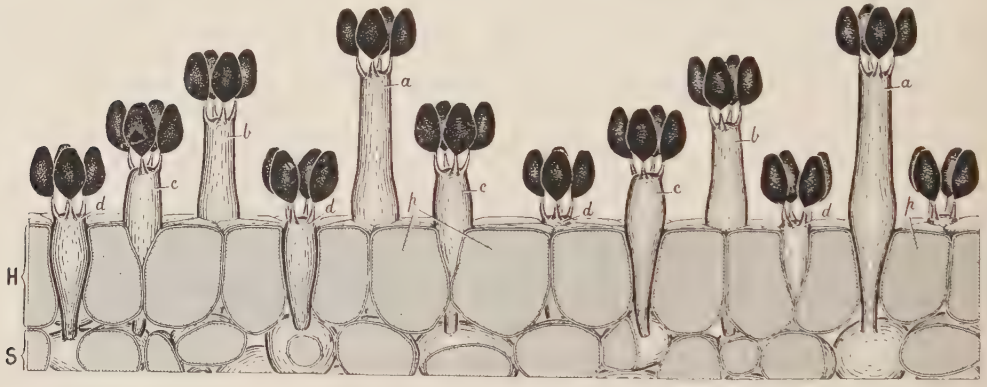


FIG. 156.—*Coprinus micaceus*. Cross-section of hymenium showing tetramorphic basidia. S, subhymenium; H, hymenium. *a a*, the first-generation basidia (the longest); *b b*, the second-generation basidia (all shorter than *a a*); *c c*, the third-generation basidia (all shorter than *b b*); *d d*, the fourth-generation basidia (all shorter than *c c* and scarcely protuberant); *p p*, paraphyses. Magnification, 77.

hymenium along with the spores. There is no evidence that the cystidia of the Coprini ever become isolated from the hymenium spontaneously. Under natural conditions their fate is simply that of autodigestion.

The contents of the cystidia of *Coprinus micaceus* attracted the attention of de Bary,[2] who described and illustrated them in 1866. He pointed out that each cystidium of a half-developed gill encloses a central more or less elongated clump of protoplasm connected by radially branched and anastomosing protoplasmic strands with the

[1] Worthington Smith, "Reproduction in *Coprinus radiatus*," *Grevillea*, vol. iv, 1875, p. 60. Also *vide supra*, p. 273.

[2] Anton de Bary, *Morphologie und Physiologie der Pilze, Flechten und Myxomyceten*, Leipzig, 1866, p. 171.

thin protoplasmic layer lining the cell-wall ; and he stated that the strands show lively amoeboid movement. My own observations, in the main, confirm those of de Bary. Young cystidia upon the gill-sides (Fig. 157, A and B) or the gill-edges (Fig. 157, D and E) exhibit a foam structure internally, for their cell-contents are divided up into a considerable number of vacuoles by thin plates of protoplasm. If one focusses the protoplasm just inside the wall of a cystidium, as shown in Fig. 157 at B, the limits of some of the vacuoles can be clearly perceived. Where the plates of protoplasm meet at the centre of each cell there is usually a clump of protoplasm which slowly changes its shape and position (Fig. 157, A, D, and E). As a cystidium grows in size, the central clump of protoplasm, as de Bary thought, appears to be suspended by protoplasmic bridles (Fig. 157, C). However, I believe that de Bary was in error in supposing that the suspension is due to the presence of bridles ; for, after watching the movements of the protoplasm for a long time, I have come to the conclusion that the central protoplasmic clump is held in position, as in very young cystidia, by thin plates of protoplasm which make up the walls of vacuoles. The cell-content of such a cystidium as that represented in optical section in Fig. 157 at C therefore appears to me to have a *foam structure* like that of soap-suds. If this view is correct, the supposed " bridles " do not traverse one great central vacuole, but are linear aggregations of protoplasm, each one formed where three protoplasmic plates meet together. The central clump of protoplasm, in the course of several minutes, moves slowly and irregularly about a more or less central cell-position and, at the same time, changes its shape. I believe that this movement is due to alterations taking place in the relative volumes of the surrounding vacuoles. I was unable to observe protoplasmic streaming of the kind that is so well known in the bridles of the epidermal cells of Flowering Plants. In vain did I endeavour to detect particles moving along the strands of protoplasm. Several times, however, I saw two strands, which together formed a V, become Y-shaped rather quickly ; but this I now believe to have been due to a rather rapid alteration in the relative size of the associated vacuoles. There can be no doubt that some kind of protoplasmic streaming must go on

in a cystidium either continuously or at irregular intervals; for, otherwise, the walls of the vacuoles could not change their dimensions. The cystidia grow apically, and, as they elongate, their

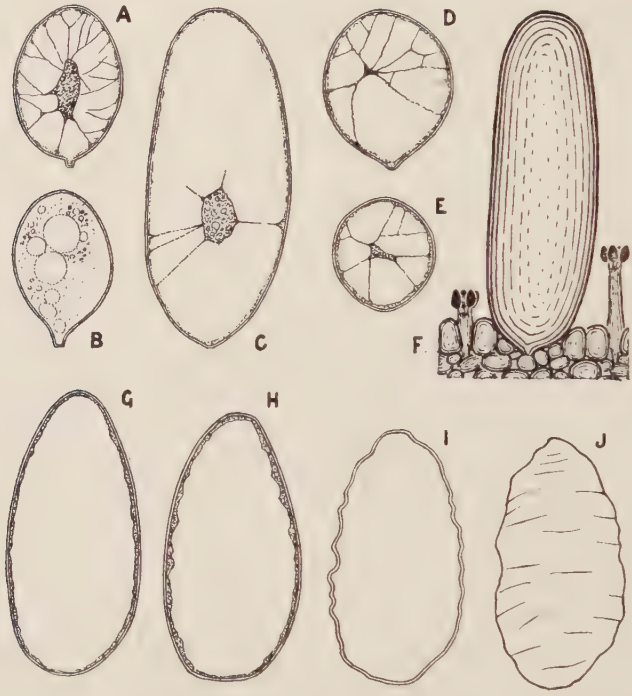


Fig. 157.—*Coprinus micaceus*. A–E, showing protoplasmic contents. A and B, two young cystidia; C, a full-grown one; all from the surface of the hymenium. D and E, cystidia from a gill-edge. In B, the vacuolated protoplasm lies just beneath the cell-wall. In A, C, D, and E, a central clump of protoplasm, which constantly moves about the middle of each cystidium, is supported by the protoplasmic walls of large vacuoles. F, a cystidium projecting from the hymenium; the hymenium also contains several paraphyses and two basidia, one of the shortest and one of the longest types. G–J, dying cystidia: as the walls contract, the protoplasm is first thrown into transverse wrinkles (G and H) and then the walls become more or less transversely folded (I and J). Magnification, 293.

protoplasmic contents diminish in quantity and the strands separating the vacuoles become fewer. In some cystidia, no doubt, the protoplasmic clump is brought to the periphery of the cell, and then one huge vacuole, like that represented in Fig. 157 at G, occupies the whole of the cell-centre.

Wettstein[1] states that the walls of the cystidia of *Coprinus micaceus* are provided with very delicate, inner, ring-shaped, wall-thickenings, which are difficult to see in fresh preparations but which can be readily observed in stained ones which have first been treated with a water-withdrawing reagent; and he supports his view with two illustrations of cystidia showing the supposed thickenings. I have looked very carefully for these thickenings in fully turgid living cystidia, but have never been able to perceive them. All the living cystidia seem to me to have an evenly thickened wall like that shown in Fig. 157 at A, B, and C. However, as a full-grown cystidium dies and collapses, wrinkles do appear upon it; but they are due, not to membrane-thickenings, but (1) to the local ridging-up of the layer of protoplasm which is applied to the cell-wall and (2) to wall-folding. In Fig. 157 at C is represented a normal living cystidium with no sign of wrinkles upon it whatsoever. Now such a cystidium, if left for a short time immersed in water, dies and becomes wrinkled. When dying, a cystidium contracts somewhat in length owing to a loss of turgidity (Fig. 157, G, H), and this leads to a ridging up of the lining layer of protoplasm transversely (Fig. 157, H). Thus, to the observer, delicate lines appear passing around the cystidium. Later on, the cystidium becomes shorter still, and then the wall becomes distinctly folded (Fig. 157, I, J). Exactly what Wettstein took for annular wall-thickenings I do not know, but I am convinced from my own observations that no such thickenings as he thought he saw occur in the cystidia of *Coprinus micaceus.*

The Discharge of the Spores.—In preparation for the beginning of the process of spore-discharge, the pileus becomes campanulate. As the pileus expands, the radial furrows in the flesh open out and the gills become well separated from one another, so that between any two adjacent gills there comes into existence a fairly wide interlamellar space (Fig. 158, C, D, E, p. 354). If one examines a fruit-body at this stage of development from below with the naked eye, one can see that the cystidia are too short to stretch across the interlamellar spaces and appear as horizontal pegs projecting

[1] Richard v. Wettstein, "Zur Morphologie und Biologie der Cystiden," *Sitzungsber. Kais. Akad. Wiss., med.-naturw. Kl.*, Wien, Bd. XCV, Abt. I, 1887, p. 15.

outwards from the gill-surfaces (*cf.* Fig. 146, p. 325). When the gills have been pulled apart in the manner just indicated, spore-discharge begins along the base of each gill, and there comes into existence a zone of spore-discharge which proceeds upwards on both sides of each gill, as in other Coprini.

The zone of spore-discharge in *Coprinus micaceus* is more complex than in any other species of Coprinus, for it consists of four sub-zones. The highest sub-zone of spore-discharge belongs to the basidia of the first generation, the next highest sub-zone to the basidia of the second generation, and so on for the third and fourth sub-zones. The zone of spore-discharge, owing to its including four sub-zones instead of two, is unusually wide, its actual width being 0·15 mm. The relations of the four sub-zones of spore-discharge to one another in *Coprinus micaceus* are of the same nature as those of the two sub-zones of *Coprinus sterquilinus*, and it will therefore be unnecessary to describe them and illustrate them in detail. It may simply be remarked that, in any narrow band of the hymenium (say 0·15 mm. wide) which is parallel to the free gill-edge, the basidia of the first generation shed their spores first, the basidia of the second generation their spores next, the basidia of the third generation their spores next, and the basidia of the fourth generation their spores last of all, *i.e.* the basidia shed their spores in succession according to their length, beginning with the longest. This permits of all the basidia, which as we have seen are crowded together on the hymenium, getting rid of their spores safely without any collision.

It was noticed that, when seen from above on a living gill, the four spores of each basidium are more divergent from the basidial axis in the zone of spore-discharge than above this zone higher up the gill. It thus appears that the divergence of the four spores of each basidium increases just before spore-discharge takes place. The significance of this fact remains to be investigated.

The spores are discharged from their basidia in the same manner as in other Hymenomycetes. The drop of water excreted at the hilum of each spore takes from five to ten seconds to attain full size. The spore and its drop always disappear from the end of the sterigma at the same moment, and it is therefore reasonable to

suppose that the drop is carried away upon the spore. Of the correctness of this view I was able to obtain conclusive evidence in one instance. I watched a drop develop on the hilum of a spore until its diameter became equal to one-half that of the spore. The spore was then shot away and, as the hymenium was turned upwards, it fell upon a neighbouring basidium. Immediately after it settled, *i.e.* less than one second after its discharge, I noticed that the drop was clinging to its upper side at one end. Very soon the drop disappeared, doubtless either by flowing around the spore or by evaporating. I have already recorded similar observations for *Coprinus sterquilinus* and for *Entoloma prunuloides*.[1]

In Fig. 158, in the series of drawings of vertical sections of fruit-bodies shown at A, B, C, D, and E, the ripening of the spores from below upwards on the gills can be traced macroscopically by the progressive darkening of the exposed gills from below upwards. The darkening of the gills is due, in the main, to the development of a brown pigment in the walls of the spores.

During spore-discharge, if we exclude a consideration of the cystidia, there are, just as in *Coprinus sterquilinus* and *C. comatus*, five zones from above downwards at the free margin of each gill: (1) a zone with perfectly ripe spores on the basidia, (2) a zone of spore-discharge, (3) a spore-free zone, (4) a zone of autodigestion, and (5) a zone along the very edge of the gill containing the products of autodigestion. The first two of these zones have been sufficiently dealt with.

The spore-free zone, like the zone of spore-discharge, is relatively larger than in most other Coprini. In one fruit-body I observed with the microscope that it was 0·1 mm. wide. However, it may often be wider still, for in some fruit-bodies which had been placed under a bell-jar I noticed that it could be seen as a fairly broad light band with the naked eye.

The zone of autodigestion is very narrow, as in other Coprini, and it passes downwards into the gill-edge zone of the products of autodigestion. Along the extreme edge of any gill which has been shedding spores for some time there is a black line which, when examined with the microscope, is found to be due to the presence

[1] These *Researches*, vol. ii, 1922, pp. 14–15.

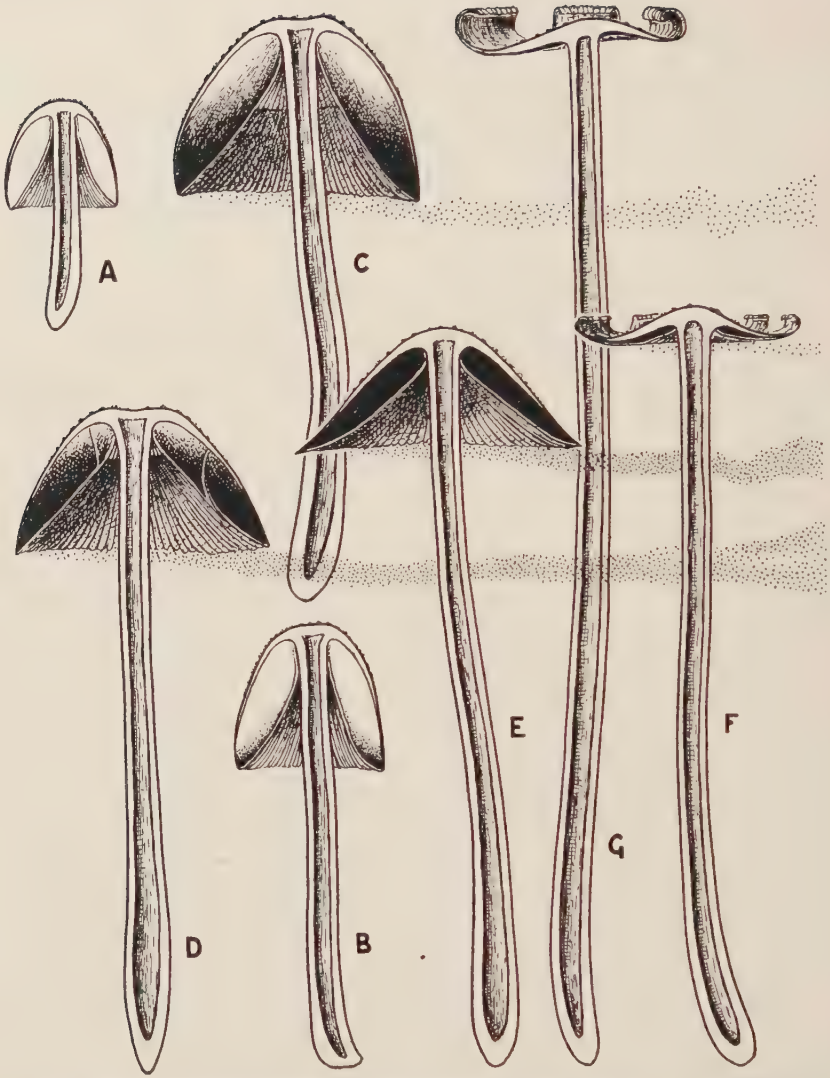


FIG. 158.—*Coprinus micaceus.* Seven stages of development in the fruit-body, as seen in vertical sections. A, very young; spores developing but unpigmented, hence gills are white. B, stipe elongating; spores ripening from below upwards on the gills. C, pileus is expanding; the spores are still ripening from below upwards on the gills; spore-discharge from the lower edges of the gills and autodigestion have begun; the carrying off of the spores by a gentle movement of the air is shown diagrammatically. D, stipe still elongating; spore-discharge from the gill-edges has attained its maximum; the gills have become reduced by autodigestion to about two-thirds their original size; spores are still ripening near the tops of the gills from below upwards. E, spore-discharge still at the maximum; the spores are now fully pigmented all over the surface of the gills; the gills have become reduced by autodigestion to about one-half their original size. F, spore-discharge is declining; the gills have become reduced to about one-twelfth their original size; the exhausted rim of the pileus is revolute and therefore does not hinder the liberation of the spores. G, a fruit-body which has just ceased to shed spores; the gills are reduced to mere ridges; the rim of the pileus is sharply revolute. In F and G, the stipes have attained their maximum length. Natural size.

in it of a large number of waste spores, which have gradually collected in the zone of the products of autodigestion as this has moved upwards.

A series of stages in the discharge of the spores and in the destruction of the gills from below upwards is shown in Fig. 158 (p. 354). The drawing A represents a very young fruit-body in which the gills are quite white owing to the fact that they do not bear any ripe spores. At B is an older fruit-body with spores ripening at the base of the gills. At C is shown a fruit-body in which the discharge of the spores has just begun along the lower margins of the gills, although toward the top of the gills the spores have not yet begun to turn brown and are therefore far from being mature. At D is a fruit-body in which spore-discharge is being carried on with the maximum activity. Here the gills have already been reduced somewhat in width by autodigestion. At E the pileus is beginning to become revolute, spore-discharge is still extremely active, and the gills have become considerably narrowed by autodigestion. At F the pileus is now quite revolute, spore-discharge is ceasing, and the gills are very much reduced in size. Finally, at G a fruit-body is shown which has an extremely revolute pileus, and in which spore-discharge has entirely ceased. The spore-streams shown diagrammatically below the pilei in C, D, E, and F, are represented as being carried away by a light current of air passing slowly from the left to the right of the Figure. The spore-discharge period, during which a pileus passes from the stage shown at C to the stage shown at G, is at least twenty-four hours in length. The upward passage of the zone of spore-discharge is rather slow relatively to that of *Coprinus sterquilinus*. However, the basidia are much smaller and more crowded together in *Coprinus micaceus* than in *C. sterquilinus*, so that a slower upward passage of the zone of spore-discharge in the former fungus is only what one might expect.

Let us now turn our attention to the cystidia and study their fate. Just before spore-discharge is about to begin, as we have seen, the cystidia project from the gills as free and mechanically functionless pegs. All the cystidia are destroyed by autodigestion and, contrary to what was once supposed, none of them fall out

from between the gills with the spores. The first cystidia to suffer destruction are those which are present along the lower parts of the gill-edges ;[1] and their cell-walls are destroyed and collapse ensues in the manner already described for the cystidia of *Coprinus atramentarius*.[2] Unless the marginal cystidia were reduced to fluid in this way, they would act as obstacles to the first spores to be discharged by obstructing their path of fall. After the destruction of the marginal cystidia, and only then, spore-discharge begins ; and the first spores to be shot away are always those situated on the basidia in a zone immediately above the edge of the gill where the destroyed marginal cystidia were located. The cystidia on the faces of the gills are destroyed by autodigestion in succession from below upwards, just as in *Coprinus atramentarius* ; and here, also, each cystidium disappears some minutes before the basidia which are immediately above it begin to discharge their spores. Thus the cystidia on the gill-sides cannot become hindrances to the fall of the spores.

Effect of the Weather on the Fruit-bodies.—The successful discharge of the spores in the manner that has been described in the preceding pages is dependent to some extent on the weather. When the air is sufficiently moist, the spores are shed and autodigestion takes place from below upwards on each gill from the bottom to the top of the pileus. However, when a fruit-body owing to transpiration loses water beyond a certain amount, the spores cease to be discharged, the basidia collapse, the process of autodigestion makes no further progress, the gills shrivel, and the pileus-flesh dries. Thus, in very dry weather, fruit-bodies are apt to dry up with all their parts well preserved although much shrunken. When such drying takes place, the beautiful mechanism for the production and liberation of the spores is rendered abortive. The millions of spores which have been produced by the basidia cling to the gills, sink down to the ground with the collapsing fruit-body, and thus fail to be dispersed by the wind.

[1] *Cf.* Fig. 118, B, *s*, p. 278.

[2] Pp. 286–289.

CHAPTER XII

THE BIOLUMINESCENCE OF PANUS STYPTICUS

Introduction—The Length of Spores of English and North American Origin—The Divergence of Imbricating Fruit-bodies—Retention of Vitality after Desiccation and during Frost—First Observations on the Bioluminescence of *Panus stypticus*—Nomenclature of the Two Physiological Forms—A Simple Mode of Demonstrating Bioluminescence with a Plant—The Phenomenon of Bioluminescence with Special Reference to Fungi—Further Observations on the Bioluminescence of *Panus stypticus luminescens*—Photographs made with the Light of the Gills—The Emission of Light by the Mycelium—A Photograph made with the Light of the Mycelium—Dependence of Luminescence on the Presence of Moisture—Dependence on Oxygen—Effect of Anaesthetics—Effect of Temperature—Evidence Proving the Existence of Luminous and Non-luminous Forms of *Panus stypticus*—Hyphal Fusions between the Two Forms—Geographical Distribution of the Two Forms—Luminous and Non-luminous Forms of *Xylaria Hypoxylon*—The Bioluminescence of Decaying Leaves—Persistence of Luminescence in Leaves—Photographs made with the Light of Luminous Leaves—Remarks on the Bioluminescence of *Armillaria mellea*

Introduction.—*Panus stypticus* is a wood-destroying fungus. Its fruit-bodies are often found in groups or imbricating clusters on the dead stumps or logs of oaks, birches, alders, and other trees both in Europe and North America (Fig. 159). I myself have gathered this Hymenomycete in England and in eastern and central Canada.

In a typical fruit-body (Figs. 160 and 161) the stipe is lateral, short, compressed from above downwards, usually ascending, dilated on approaching the pileus, and paler than the gills ; while the pileus is 0·5–1·5 inches in diameter, reniform, thin but soft, cinnamon becoming pale, and with the cuticle on its upper surface broken up into minute scurfy squamules. The gills end sharply at the top of the stipe and are therefore not decurrent, and they are 1–1·5 mm.

deep, thin, crowded, beautifully connected by veins, and cinnamon in colour.

The spores are remarkably small. According to Massee [1] they measure only 3 × 1·5–2 μ, according to Miss Johnson [2] 4 × 3 μ, and according to Kauffman [3] 4–5 × 2 μ. The small size of the

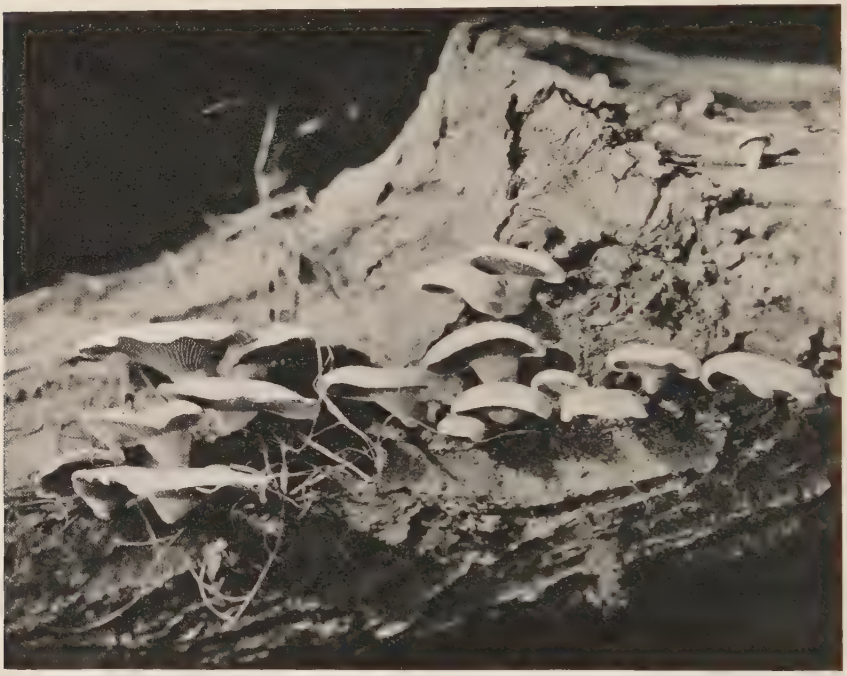


Fig. 159.—*Panus stypticus* physiological form *non-luminescens*. A group of fruit-bodies projecting from a stump at Tanworth-in-Arden, Warwickshire, England. Photographed by Jessie S. Bayliss Elliott. Natural size.

fruit-bodies and the crowding and thinness of the gills are doubtless correlated in part with the very small size of the spores and of the basidia which bear them. A fruit-body of *Panus stypticus* both macroscopically and microscopically is, as it were, a miniature of a fruit-body of *Pleurotus ulmarius* or *P. ostreatus*.

The fruit-bodies of *Panus stypticus* are hot to the taste and have

[1] G. Massee, *British Fungus-Flora*, vol. ii, 1893, p. 309.

[2] E. M. Johnson, "On the Biology of *Panus stypticus*," *Trans. Brit. Myc. Soc.*, vol. vi, 1920, p. 348.

[3] C. H. Kauffman, *The Agaricaceae of Michigan, U.S.A.*, vol. i, 1918, p. 48.

an astringent action on the palate and throat. They often contain a certain amount of tannin, as may be proved by the blue reaction given with certain iron salts; but this tannin, here as in other lignicolous agarics, appears not to be manufactured by the fungus during its metabolic activity but merely to be absorbed from the substratum upon which the fungus grows.[1] Bourquelot found that some fruit-bodies which he analysed contained 1·6 per cent. trehalose and traces of mannite and grape-sugar.[2] Rosoll extracted from the fruit-bodies a brown pigment which is soluble in alcohol and ether and turns a dirty red with sulphuric acid. The alcoholic solution has a green fluorescence.[3]

The Length of Spores of English and North American Origin.—Fruit-bodies of *Panus stypticus* were collected at: (1) Great Missenden, England; (2) West Malvern, England; (3) Ann Arbor, Michigan, U.S.A.; (4) Saint Paul, Minnesota, U.S.A.; and (5) Ottawa, Ontario, Canada. After revival in the laboratory, they all gave rise to copious spore-deposits. At my request, Mr. W. F. Hanna, using the very accurate plate-micrometer method already described in Volume I, measured the lengths of the spores in all the five spore-deposits. As a result of measuring 100 spores in each deposit, he found that the average length of the spores was: for (1) a Great Missenden fruit-body, 5·3 μ; for (2) a West Malvern fruit-body, 5·3 μ; for (3) an Ann Arbor fruit-body, 5·0 μ; for (4) a Saint Paul fruit-body, 5·2 μ; and for (5) an Ottawa fruit-body, 5·1 μ. Thus the variation in the average length of the spores for all these five fruit-bodies taken together was only 5·0–5·3 μ. It is clear, therefore, that no varietal distinction between the English and North American fruit-bodies could be made on the basis of spore size.

The Divergence of Imbricating Fruit-bodies.—It not infrequently happens that the fruit-bodies of *Panus stypticus* are caespitose, and then the individual fruit-bodies of a single cluster diverge from one another. This is well shown in the vertical section represented in Figure 161. Here, from above downwards, the first stipe ascends at an angle of about 65°, the second ascends at an

[1] *Vide* Julius Zellner, *Chemie der Höheren Pilze*, Leipzig, 1907, p. 134.
[2] *Ibid.*, p. 103. [3] *Ibid.*, p. 169.

angle of about 25°, the third descends at an angle of about 20°, and the fourth descends at an angle of about 70°. The pilei all tend to take up a more or less horizontal position. The result of these divergences is that the three highest fruit-bodies, although imbricating, all have a free space beneath their pilei sufficiently deep to permit of the wind carrying away the spores falling from the gills before they can settle on the tops of the subjacent pilei. The vertical distance between each pair of pilei is only 4–10 mm. However, the spores are so small that they must fall in still air at a rate of less than 0·5 mm. per second.[1] Assuming the rate of fall to be 0·5 mm. per second it is clear that, in still air, the spores of the three highest pilei would fall for 8–20 seconds before settling on the pilei below. A simple mathematical calculation shows that, if the pilei are assumed to be one inch in diameter, a horizontal air-current passing between the pilei at the rate of only one foot per minute would be much more than sufficient to carry away all the

FIG. 160.—*Panus stypticus* physiological form *luminescens* a luminous fungus. Fruit-bodies separated from an imbricating mass. The top one shows the upper surface, the two middle ones show the lower surface, and the bottom one is seen in section. The fruit-bodies were kept dry for six weeks and then revived by wetting their upper surfaces. They then discharged great numbers of spores and at the same time exhibited luminescence. The lower sides of the pilei glowed more strongly than the upper. Obtained from a stump at Ottawa, Ontario, Canada. Natural size.

[1] This statement is based on comparative observations of the rates of fall of spores of various sizes. *Vide* these *Researches*, vol. i, 1909, p. 175.

spores falling from the three upper fruit-bodies before they would have time to settle on the tops of the pilei below. The manner in which the spores are carried away from the pilei by the wind is illustrated diagrammatically in Fig. 161.

The divergence of caespitose or much crowded fruit-bodies is a phenomenon not peculiar to *Panus stypticus*, but one which may be observed in *Polyporus squamosus*, *Lentinus lepideus*, and many other Hymenomycetes.[1] Its exact causes require elucidation. In the three species just named the divergence seems to be due, firstly, to the fact that the stroma upon which the fruit-bodies arise bulges out more or less hemispherically from the substratum and, secondly, to the fact that the stipes of the individual fruit-bodies, which when very young are ageotropic, grow out in a direction which is perpendicular to the surface of the stroma. The more or less horizontal position taken up by the pilei, when these come to be developed, is doubtless due to geotropic stimuli. The divergence of the fruit-bodies in clusters of *Coprinus micaceus*, *Armillaria mellea*, and *Pleurotus ostreatus*, etc., growing naturally in the open, and of *Psalliota campestris* growing in beds of manure may be due to mechanical pressure alone or, possibly, to a reaction to mutual contact stimuli. From the functional point of view the phenomenon of fruit-body divergence, wherever it occurs and however it is caused, is of high importance, for it is a prime factor in providing those spaces between adjacent fruit-bodies which are required to enable the wind to carry away the spores and thus bring about the dissemination of the species.

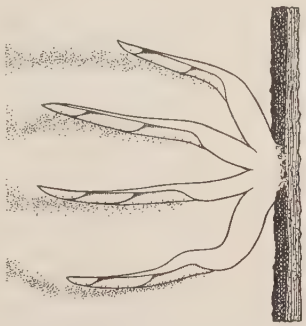


FIG. 161.—*Panus stypticus* p.f. *luminescens*. Vertical section through four fruit-bodies forming an imbricating cluster attached laterally to a tree. To show the divergence of the fruit-bodies from one another and the spore-clouds being carried away by the wind. Obtained at Montreal, Province of Quebec, Canada. Natural size.

Retention of Vitality after Desiccation and during Frost.—During moist weather the pilei of *Panus stypticus* are flattened and

[1] *Cf.* these *Researches*, vol. i; for *Polyporus squamosus*, p. 57, also Plate V, Figs. 31, 32, 36; for *Lentinus lepideus*, p. 48, also Fig. 16, A.

more or less horizontally extended ; and in this state, as is shown by a study of successive spore-deposits, they liberate immense numbers of their very minute spores. In dry weather, however, their edges curl downwards and inwards to such a degree that the gills become almost entirely covered up (Fig. 162). This covering of the hymenial surface of the pileus also occurs in *Schizophyllum commune*, *Trogia crispa*, and some other small lignicolous fungi. Whether or not it has any ecological significance in so far as protection of the hymenium from mechanical injuries is concerned, is uncertain. It may be that the rolling up of the fruit-bodies in dry weather, like the rolling up of the leaves of certain Ericaceae and Gramineae under similar conditions, is significant only in so far as it is a means of diminishing the surface exposed and thus a means of bringing about reduced transpiration.

When the upper surface of the pileus of a dried fruit-body is moistened either naturally by rain-drops or artificially with wet cotton wool, water is quickly absorbed through the squamulose cuticle and, in the course of about an hour, the pileus uncurls and becomes fully extended. In one experiment, made with material that had been kept dry for a few weeks, spore-discharge began to take place within 2·5 hours of the time when the pilei were first wetted.

Dried fruit-bodies of *Panus stypticus* can retain their vitality for several months. Some fruit-bodies were kept dry in the laboratory from November 11 until May 10, *i.e.* for a period of six months. At the end of this time they were allowed to absorb water, whereupon they quickly revived and soon began to shed spores. The white spore-deposits formed beneath the pilei were very copious and the spores, upon being placed in nutrient agar, soon germinated. This confirms a similar experiment made by Miss Johnson.[1]

Some fruit-bodies, which had been gathered in the open and left to dry, after being revived with water in the laboratory, shed spores for a period of 10 days. Doubtless, in nature, the spore-discharge period extends over at least two weeks. In the open the discharge of spores must sometimes be temporarily interrupted by drought, only to be resumed when wet weather supervenes.

The fruit-bodies of *Panus stypticus* can successfully withstand

[1] E. M. Johnson, *loc. cit.*, p. 351.

not only a high degree of desiccation but also intense frost. They soon recover after having been subjected for a night to a temperature of −10° C. Frost, as well as drought, may therefore temporarily interrupt the production and liberation of spores. The resistance of the fruit-bodies of *Panus stypticus* to frosts which are

Fig. 162.—*Panus stypticus* physiological form *luminescens*. Fruit-bodies obtained in dry weather, with edges of the pileus curled downwards and inwards, thus partially covering up the hymenial surface. A, imbricating fruit-bodies on a piece of wood from the side of a stump. B, fruit-bodies seen from above. C, fruit-bodies seen from below. Collected at Ottawa by W. S. Odell. Photographed by the Photographic Division of the Geological Survey of Canada. Natural size.

fatal to many soft-fleshed agarics accounts to some extent for the fact that this fungus flourishes late in the autumn.

It is probable that, in the colder parts of Canada and the United States, owing to an interruption caused by the intense cold of the mid-winter months, the spore-discharge period of *Panus stypticus*, like that of *Schizophyllum commune*,[1] *Daedalea*

[1] At Winnipeg, where the winter is long and comes and goes rather suddenly, the fruit-bodies of *Schizophyllum commune* often become frozen before they are exhausted. Such frozen fruit-bodies, when brought into a warm laboratory in mid-winter, quickly thaw and, if kept moist, soon begin to emit a cloud of spores. I have no doubt that such fruit-bodies, under natural conditions, shed some of their spores in the autumn and the remainder in the following spring. *Cf.* these *Researches*, vol. i, p. 127.

confragosa,[1] and some other lignicolous Hymenomycetes, is often broken into two parts in such a way that some of the spores are shed in October or November and the others in March or April.

First Observations on the Bioluminescence of Panus stypticus. —In 1901, Atkinson,[2] describing *Panus stypticus*, said: "When freshly developed the plant is phosphorescent." Molisch,[3] in 1904, in a work which he published on light-producing plants, mentioned *Panus incandescens* (which grows in Australia) as being luminescent, but not *P. stypticus*.

In 1910, with a view to investigating the alleged "phosphorescence"[4] of *Panus stypticus*, I procured some freshly-grown fruit-bodies of this species from a stump at Montreal, allowed them to dry up, took them to Winnipeg and there, six weeks after they had been gathered, moistened the upper surfaces of their pilei with wet cotton wool.

Six and a half hours after the fruit-bodies had been moistened, they were found to have revived. The pilei were much swollen, the gills fully extended, and great numbers of spores were being discharged by the hymenium. On taking the revived fruit-bodies into a dark room, I perceived at once that they were strongly luminescent. The luminescence was most brilliant on the under surface of the pileus where the gills were developing and setting free their millions of spores; but the upper surface also gave out light, although less strongly. The shedding of spores and the exhibition

[1] These *Researches*, vol. ii, 1922, pp. 116–117.

[2] G. Atkinson, *Studies of American Fungi. Mushrooms, edible, poisonous, etc.*, Ithaca, U.S.A., 1901, p. 136.

[3] H. Molisch, *Leuchtende Pflanzen*, Jena, 1904, pp. 28–29.

[4] The word *phosphorescence* has been used in a loose manner to indicate all kinds of luminescence, particularly that of phosphorus and of luminous animals and plants. For physicists, phosphorescence means the absorption of radiant energy by substances which afterwards give out the energy as light. For animals and plants we ought therefore to speak not of phosphorescence but of *luminescence*. There is no reason to suppose that the luminescence of plants and animals is caused in these organisms by the presence of the element phosphorus. For a classification of luminescences, according to the means of exciting the light, *vide* E. N. Harvey, *The Nature of Animal Light*, Philadelphia and London, 1920, p. 23. Chemiluminescence is the production of light during a chemical reaction at low temperatures. Oxygen is required for the process. Bioluminescence and chemiluminescence are essentially similar phenomena.

of luminescence continued for from 10 to 14 days. At the end of this time, the fruit-bodies began to go mouldy.

At my request, Mr. W. B. Grove was good enough to send me two lots of fruit-bodies of *Panus stypticus* which he had gathered in England. One lot had been kept dry for five years, whilst the second lot had been recently gathered in the month of December. Upon arrival in Winnipeg, all the fruit-bodies were moistened upon their upper surface with wet cotton wool in the usual manner. The five-year-old fruit-bodies failed to revive and soon went mouldy : they had evidently lost their vitality. Of the recently gathered fruit-bodies, one did not uncurl properly, a second came to have wet discoloured gills, and a third revived in a normal manner. The first two fruit-bodies neither shed spores nor became luminescent, and had evidently lost their vitality. The third was still living, for it resumed its spore-liberating function, although not very vigorously ; but it never gave out any light and soon died. There seems to be no doubt that these three fungi, which were gathered, as already stated, in December, were old and practically exhausted before being removed from their substratum.

The observations just recorded taught me that *Panus stypticus* as it occurs in North America is a luminous fungus, but left me in doubt whether *Panus stypticus* as it occurs in Europe gives out light or not.

Since 1910, from investigations made upon several collections of the fungus gathered in North America and England, I have come to the following conclusions : (1) the mycelium and fruit-bodies of *Panus stypticus* of North American origin are luminous, and (2) the mycelium and fruit-bodies of *Panus stypticus* of English origin are non-luminous. The evidence supporting these conclusions will be adduced in a subsequent Section. In the meanwhile the reader should bear in mind that, whenever the luminosity of *Panus stypticus* is mentioned, it is the North American and not the English strains of the fungus that the author has in mind.

Nomenclature of the Two Physiological Forms.—Of the species *Panus stypticus* there are evidently two forms, morphologically alike but physiologically different, occupying separate geographical areas and kept apart by the great barrier of the Atlantic Ocean.

To distinguish them from one another we are obliged to make use of a trinomial nomenclature. I therefore propose to call the luminous North American form *Panus stypticus* physiological form *luminescens*, and the non-luminous English form *Panus stypticus* physiological form *non-luminescens*. In what follows, for the sake of brevity, these names will be written *Panus stypt. luminescens* and *Panus stypt. non-luminescens* respectively.

A Simple Mode of Demonstrating Bioluminescence with a Plant.—Knowledge of the fact that freshly developed fruit-bodies of the North American strains of *Panus stypticus* are luminescent can be utilised in providing a means for demonstrating the luminescence of a plant in the laboratory at any time desired and by the simplest means. For this demonstration, it is only necessary to procure some young fruit-bodies of *Panus stypticus* from Canada or the United States in the autumn, to let them dry, to store them in a bottle and, a few hours before the demonstration is to be made, to revive them by wetting their upper surface with wet cotton wool. Then, if the fruit-bodies so revived are placed in a dark room, their luminescence becomes at once apparent.

The Phenomenon of Bioluminescence with Special Reference to Fungi.—In 1921, eleven years after making the first observations already recorded, I resumed my study of the emission of light by *Panus stypt. luminescens*. I succeeded in making photographs with the fungus light, discovered that the mycelium is luminous, and elucidated the relations of the luminescence to oxygen, water, temperature, etc. However, before communicating the results of this new investigation in detail, I shall first treat of the general phenomenon of bioluminescence with special reference to fungi.

On p. 367 is a list of Hymenomycetes which are known to give out light.

The Table shows that, up to the present, light-producing fruit-bodies have been found only in Clitocybe, Panus, and Pleurotus, the first genus being represented by one luminous species, the second by two, and the third by twelve.[1]

[1] For references to the literature, *vide* W. Zopf, *Die Pilze*, 1890, pp. 195–197; H. Molisch, *loc. cit.*, pp. 25–40; and S. Kawamura, "Studies on the Luminous Fungus, *Pleurotus japonicus* sp. nov., *Journ. Coll. Sci. Tokyo*, vol. xxxv, 1915.

Hymenomycetes Known to be Luminescent.

No.	Species.	Part luminescent.	Where found.
1	Clitocybe illudens Schw.	Fruit-body and mycelium	North America.
2	Panus incandescens B. et Br.	Fruit-body	Australia.
3	Panus stypticus Fr., p. f. luminescens	Fruit-body and mycelium	North America.
4	Pleurotus candescens Müll. et Berk.	Fruit-body	Victoria and New South Wales.
5	Pleurotus facifer B. et C.	Fruit-body	Pennsylvania, U.S.A.
6	Pleurotus Gardneri Berk.	Fruit-body	Brazil.
7	Pleurotus igneus Rumph.	Fruit-body	Island of Amboyna.
8	Pleurotus illuminans Müll. et Berk.	Fruit-body	Australia.
9	Pleurotus japonicus Kawam.	Fruit-body	Japan.
10	Pleurotus Lampas Berk.	Fruit-body	Swan River, Australia.
11	Pleurotus nidiformis Berk.	Fruit-body	Swan River, Australia.
12	Pleurotus noctilucens Lév.	Fruit-body	Manila, P.I.
13	Pleurotus olearius DC.	Fruit-body	Southern Europe.
14	Pleurotus phosphoreus Berk.	Fruit-body	Tasmania, Australia.
15	Pleurotus Prometheus B. et C.	Fruit-body	Hong Kong.
16	Armillaria mellea Vahl.	Mycelium and rhizomorphs	Europe and North America.
17	Collybia tuberosa Bull.	The germinating sclerotium	Europe and North America.
18	Collybia cirrhata Pers.[1]	The germinating sclerotium	Europe and North America.

The following species[2] have been supposed by various observers to be luminescent, but whether or not they emit a light of their own can only be decided by a number of further critical investigations: *Fomes annosus*, *Polyporus sulphureus*,[3] *P. citrinus*

[1] Apparently synonymous in part with *Marasmius sclerotipes* Bres. *Vide* Carleton Rea, *British Basidiomycetae*, Cambridge, 1922, p. 528.

[2] These are cited by Hans Molisch, *loc. cit.*

[3] W. G. Smith, in 1871, in a brief note called "Luminous Fungi" (*Journal of Botany*, vol. ix, 1871, p. 176) states that two years previously he received from a Cardiff coal mine specimens of *Fomes annosus* which were luminous and could be seen in the dark at a distance of twenty yards. And he adds: "I have also seen *Polyporus sulphureus* phosphorescent, and Mr. Broome has met with a luminous Corticium. I have heard that *C. coeruleum* is sometimes luminous." No one, so far as I know, has corroborated these statements. Up to the present, I have been unable to perceive any luminescence in the fruit-bodies of either *Fomes annosus* or *Polyporus sulphureus* and, until some one succeeds where I have failed, I may perhaps be pardoned for regarding Smith's bare statement that these two species are luminous with some degree of scepticism. It is desirable that other mycologists should examine the fruit-bodies and mycelium of the species in question in the dark and report their

(*P. caudicinus*), *Trametes pini*, *Corticium coeruleum*, and *Collybia longipes*.[1]

Probably the fruit-bodies of *Panus incandescens* and possibly the germinating sclerotia of *Collybia tuberosa* and *C. cirrhata* resemble the fruit-bodies of *Panus stypt. luminescens* in drying without loss of vitality and in exhibiting luminescence after revival.

The intensity of the light emitted by the fruit-bodies of certain Hymenomycetes is truly remarkable, and it may perhaps be best indicated by the following description of the luminescence of *Panus incandescens* as given by Lauterer.[2] This author says: "Specifically Australian is the large, white, splendidly luminous, lamellate fungus *Panus incandescens*, which commonly occurs throughout the whole of east Australia in clusters about the trunks of trees. During the day it is in no way striking, for it much resembles the larger white Agaricus species which grow in Germany. But when one goes into the bush at night and the fungus pours forth its emerald-green light—a light in the immediate neighbourhood of which one can read quite well—this delightful fairylike apparition wins our interest at once."

Gardener and Gunning were able to read by the light emitted from the fruit-bodies of the Australian and Tasmanian fungus, *Pleurotus phosphoreus*;[3] and Pfeffer[4] has recorded that on dark nights he was able to perceive the glow from a strongly luminous fruit-body of the southern European *Pleurotus olearius* at a distance of one thousand paces.[5]

findings, with a view to deciding once and for all whether or not Smith was in error in making his report.

[1] I have failed to detect any luminescence in the fruit-bodies of *Collybia radicata*, *Panus torulosus*, and *Pleurotus ostreatus*.

[2] J. Lauterer, *Australien und Tasmanien*, Freiburg i. Br., 1900, p. 212. I have translated the passage from the German as quoted by Molisch (*loc. cit.*, pp. 28–29).

[3] *Vide* W. Zopf, *Die Pilze*, Breslau, 1890, p. 197.

[4] W. Pfeffer, *Pflanzenphysiologie*, Aufl. I, 1888, Bd. ii, p. 419.

[5] Luminous Fungi occurring in woods sometimes cause fear and, in the minds of the ignorant and superstitious, have even formed a basis for ghost stories. Of this I myself had evidence in Manitoba. In Western Australia James Drummond once found a very large luminous fungus which was 16 inches in diameter and hung it up in a sitting room. Then he relates: "We called some of the natives and showed them the fungus when emitting light, and the poor creatures called out 'chinga,' their name for a spirit and seemed much afraid of it" (Cooke and Berkeley, *Fungi, their Nature, Influence, and Use*, London, ed. III, 1882, p. 110).

The colour of the light emitted by luminous Hymenomycetes is of more than one kind. In some species, *e.g. Pleurotus japonicus*, it is white; in others, *e.g. Panus incandescens*, green; in others, *e.g. Panus stypt. luminescens*, greenish-white; in others, bluish-green, etc. In no species at present known is it red.[1] In the common *Armillaria mellea* it is white but with a faint tinge of bluish-green.[2]

Spectrum analysis has shown that the light given out by luminous fungi and bacteria differs in its wave-lengths in different species, but that in these organisms, as in animals, the spectrum is a continuous one.[3] Ludwig,[4] in 1884, found that the spectrum of *Armillaria mellea* stretches from about the middle of the yellow region, through the green region, and well into the blue region (wave-length 0·54–0·46 μ); and Molisch,[5] in 1904, found that the spectrum of *Mycelium X* (belonging to an unknown species) is rather narrow, coincides practically with the yellow and green regions, and is brightest in the green region (wave-length 0·57–0·48 μ).

The light emitted by luminous fungi doubtless resembles that of the Fire-fly and the Glow-worm in not being accompanied by the production of any appreciable amount of heat. Bioluminescent organisms in their production of "light without heat" are, from the illuminating engineer's point of view, veritable marvels of efficiency.[6]

In 1915, Kawamura[7] published an account of his studies of a Japanese luminous fungus, *Pleurotus japonicus*, and his observations were as follows. The fruit-bodies somewhat resemble those of *P. ostreatus* and are found in imbricating clusters upon the decaying

[1] However, there is a Fire-fly, *Photinus pyralis*, which gives out a reddish glow. *Vide* E. N. Harvey, *loc. cit.*, p. 44.

[2] *Cf.* H. Molisch, *loc. cit.*, p. 122.

[3] *Vide* E. N. Harvey, *loc. cit.*, pp. 46–47, also Fig. 9.

[4] F. Ludwig, "Über die spektroskopische Untersuchung photogener Pilze," *Zeitschr. f. wiss. Mikroskopie*, Bd. I, 1884, pp. 181–190.

[5] H. Molisch, *loc. cit.*, p. 132. Molisch, in his Fig. 13, shows comparative illustrations of the spectra of sunlight, *Bacterium phosphoreum*, *Mycelium X*, and the beetle, *Pyrophorus noctilucus*.

[6] For a full discussion of this subject *vide* E. N. Harvey, *loc. cit.*, pp. 40–66.

[7] S. Kawamura, "Studies on the Luminous Fungus, *Pleurotus japonicus* sp. nov.," *Journal of the College of Sci., Imper. Univers. of Tokyo*, vol. xxxv, 1915, Art. 3, pp. 1–29, Plates I–III.

trunks of Beech trees (*Fagus sylvatica* var. *Sieboldi*) in autumn

Fig. 163.—*Pleurotus japonicus*, a fungus with luminescent gills. The imbricating fruit-bodies are growing from the top of a dead and rotten Beech trunk. Photographed in Japan by S. Kawamura. Reduced to $\frac{1}{6}$ natural size.

(Fig. 163). They are very poisonous and, in consequence of their

having been confused with the fruit-bodies of *P. ostreatus*, they have been the cause of numerous cases of poisoning, resulting in many deaths. The light is emitted by the gills only. The hymenium, subhymenium, and trama are all luminous, but no light is emitted by the spores. A fruit-body with a luminous area of 100 square cm. gives sufficient light in the dark to enable one to see Roman letters about 8 mm. wide, and the light can be well perceived at a distance of at least 30 metres. The light is white, not greenish, bluish, or yellowish, as in most fungi. Photographic images of the luminous surface of a fruit-body, taken in a dark room with an exposure of 7·5 or even 24 hours, were all very faint; but, when leaves were placed between the under side of a pileus and a very sensitive photographic dry-plate,[1] with an exposure of nearly two hours, some excellent dark prints on a white background (Fig. 164) were secured. The minimum temperature for the emission of light is 3°–5° C., the maximum 40° C., and the optimum about 10°–15° C. When the fruit-body is suddenly immersed in nitrogen gas, the luminescence begins to fade after 10 seconds, becomes very feeble after 50 seconds, is scarcely recognisable after 1 minute 20 seconds, and finally becomes completely invisible after 1 minute and 40 seconds. When a fruit-body is plunged into hydrogen gas, the luminescence begins to fade after 10 seconds and becomes invisible after 30 seconds.[2] Chloroform vapour causes the light to vanish in 55 seconds and ether vapour in 1 minute 50 seconds. If, after being immersed in ether vapour for 1 minute 50 seconds, the fruit-body is placed in pure air, the luminescence returns after 30 seconds. Exposure of a fruit-body to oxygen gas causes no change in the intensity of the light. In oxygen, therefore, the fungus behaves just as it does in air.

In England there are no Agaricineae in which the pileus is luminous, but in Canada and the United States there are two: *Panus stypticus luminescens* and *Clitocybe illudens*.

Clitocybe illudens, in North America, is popularly known as *Jack-*

[1] "Ilford Alliance, fastest."

[2] Kawamura told me in a personal interview that "thirty minutes" in his text was a misprint for "thirty seconds." In citing his results I have therefore introduced the correction.

my-lantern owing to its luminescence. Its fruit-bodies (Fig. 165) are large, caespitose, and saffron-yellow. Murril[1] remarks: "This species is readily recognised by its large size and brilliant colouring. It occurs throughout the eastern United States from midsummer

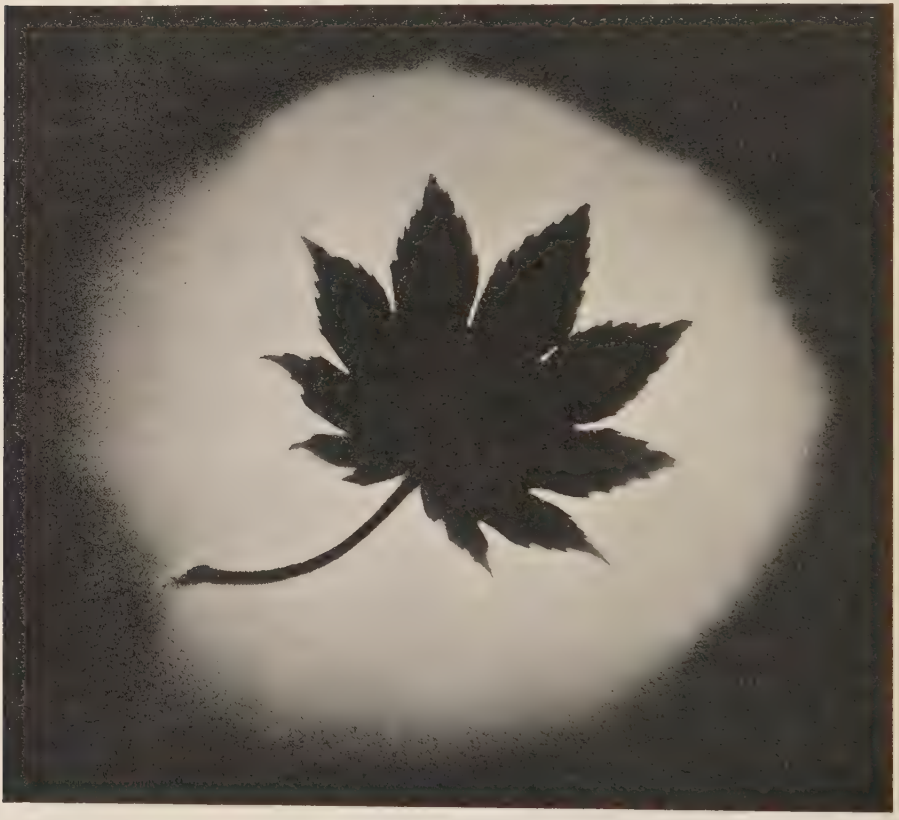


Fig. 164.—A shadow-photograph of a Maple leaf, made with the light given off by the gills of a fruit-body of *Pleurotus japonicus*. Exposure, 1 hour 50 minutes. Photographed by S. Kawamura. Natural size.

to autumn in large clusters about dying trunks and stumps of deciduous trees. On dark nights, these clusters and also dead wood containing the mycelium are usually conspicuously phosphorescent. The plant is distinctly poisonous, showing a muscarin reaction on the nerves of the heart, and producing nausea, vomiting, and

[1] W. A. Murril, "Illustrations of Fungi," *Mycologia*, vol. vii, 1915, pp. 115–116.

diarrhœa." *C. illudens* appears to be absent from central Canada, but it is known to occur in eastern Canada. Whilst at Toronto Dr. J. H. Faull once showed me a splendid cluster of living fruit-bodies which he had collected in a near-by wood.

Murril[1] examined for luminosity some fruit-bodies of *Clitocybe illudens*, as well as wood containing the mycelium, on two successive days, and noted that he was obliged to remain in the dark for some time before he could perceive any luminescence : on August 21, at 1.30 P.M. for 7 minutes, at 7.30 P.M. for 1·5 minutes ; on August 22, at 9 A.M. for 10–15 minutes, at 9 P.M. for 1–2 minutes. These facts, upon which Murril makes no comment, are easily understood on the supposition (1) that the luminous fruit-bodies, like those of *Panus stypt. luminescens*, etc., give out, both in daylight and in darkness, a steady continuous light, and (2) that the variable factor is the physiological condition of the eyes of the observer. The length of time one is obliged to wait in a dark room before observing the soft glow of a luminous fungus depends simply upon the sensitiveness of the eyes for the perception of relatively very feeble rays of light. The longer one has been exposed to strong daylight, the longer one is obliged to remain in the dark-room before perceiving luminescence, and *vice versa*. In the morning when the sun is up, especially in summer-time, one's eyes lose rapidly and in a high degree their adaptation for seeing weak lights in the dark ; whilst in the evening, when the sun is declining, or has declined, below the horizon, one's eyes are regaining, or have regained, their sensitiveness. Hence, as a rule, one perceives luminous fungi in the dark more rapidly in the evening than in the morning or at noon.

It will be shown in a later Section that the mycelium of *Panus stypt. luminescens* is luminous and gives out a very steady and continuous light for many successive weeks or months. Using Birch-block cultures as fungus lamps (*cf.* Fig. 170, p. 391), I made a series of experiments to determine the length of time it was necessary for me to remain in the dark-room before perceiving the light emitted by the fungus. The experiments were all carried out at Winnipeg on April 17, 1923.

(1) I arose at 4 A.M. and, while it was still dark, walked to the

[1] W. A. Murril, "Luminescence in Fungi," *Mycologia*, vol. vii, 1915, pp. 132–133.

University of Manitoba. Upon arrival there at 4.50 A.M. Jupiter still hung aloft the western sky, while Aurora, the rosy-fingered,

FIG. 165.—*Clitocybe illudens*, known as *Jack-my-Lantern*, a rich saffron-yellow agaric occurring throughout the eastern United States and in eastern Canada in large clusters about dying trunks and stumps of deciduous trees. At night its gills are strongly luminescent. It has a muscarin reaction on the nerves of the heart and causes nausea, vomiting, and diarrhoea. ⅔ the natural size, often much larger. From the late G. F. Atkinson's *Mushrooms, Edible and Poisonous, etc.* By courtesy of Henry Holt and Co.

was approaching in the east. Upon going into the dark-room my well-rested eyes *at once perceived the full glow of the fungus lamps*; and, even when the dark-room door was opened so as to admit the entry of a certain amount of twilight, the bioluminescence was still distinguishable.

(2) Between 5 A.M. and 5.30 A.M. I took a walk in the open. During this period of half an hour, daylight was breaking and my eyes were subjected to diffused white light. At 5.31 A.M., I re-entered the dark-room and found that before the fungus lamps could be perceived *one minute elapsed.*

(3) Between 5.45 A.M. and 6.30 A.M. I again took a walk in the open. By this time, the sun was up and from a clear and cloudless sky was shining brilliantly. After having thus been exposed to bright sunlight for 45 minutes, I re-entered the dark-room, with the result that before the fungus lamps could be perceived 9 *minutes elapsed.*

(4) At noon, between 11.45 and 12.44, I took another walk in the open, thus exposing myself to bright sunlight for an hour. At the end of this time I re-entered the dark-room and observed that before my eyes could appreciate the presence of the fungus lamps 11·5 *minutes elapsed.*

(5) In the afternoon, between 3.35 and 4.15 P.M., I took a walk in a direction in which my face was toward the declining sun. At the end of this time I re-entered the dark-room and observed that before the presence of the fungus lamps could be observed 10·6 *minutes elapsed.*

(6) In the evening, between 5.40 and 6.8 P.M., I read a book by the light coming in from a western window. At the end of this time I re-entered the dark-room and observed that before the fungus lamps could be perceived 6·5 *minutes elapsed.*

(7) Later in the evening, after dining, I walked to the University in the dark, my eyes thus becoming well rested. Upon arrival at 8.30 P.M., I went into the dark-room and *at once I saw all the fungus lamps glowing with their full intensity*, just as at 4.50 A.M. in the morning.

When going into the dark-room at the close of the fifth experiment, I was joined by my colleague, Mr. C. W. Lowe. He had been sitting in a room with the blinds down, whereas I had been exposed to bright sunlight. As soon as the door of the dark-room was closed, he at once perceived the light from the fungus lamps, whereas I could see nothing until 10·6 minutes had elapsed. On other occasions when Mr. Lowe's eyes had been exposed to bright

daylight while mine had been rested, I perceived the light from the fungus lamps much more quickly than he could.

The above series of experiments shows very clearly that the length of time one needs to spend in the dark-room before perceiving the light given out from a luminous fungus depends on the sensitiveness of the eyes to weak illumination or, in other words, on their adaptation to darkness, and varies roughly from 0 to 15 minutes. A recognition of these facts is of some interest, for there can be but little doubt that certain mycologists have failed to see the light given out by a luminous fungus taken into a dark-room because they did not wait in the dark-room sufficiently long to allow their eyes to acquire the necessary sensitiveness to feeble illumination. In testing a new fungus for luminosity, one ought to carry on observations in the dark-room for at least 15 minutes.

The fruit-bodies of *Panus stypt. luminescens* are very small compared with those of *Pleurotus japonicus* or *Clitocybe illudens*. Nevertheless, in nature, the light which they give out is not inconsiderable and may make them conspicuous at night. Dr. G. R. Bisby has informed me that once in Minnesota, when walking in a wooded place in the dark, he discovered a cluster of the fruit-bodies simply by having his attention drawn to them by the light which they emitted.

The only plants known to produce light are certain Bacteria, certain Peridinieae, certain strains of the Ascomycete *Xylaria Hypoxylon*,[1] and the Hymenomycetes enumerated above. There do not appear to be any luminescent Phycomycetes, Gastromycetes, or Fungi Imperfecti.

Luminescent bacteria are very widely distributed and about thirty species are already known.[2] They cause the luminescence often seen at the surface of damp bones, butcher's meat, sausages, marine fish, the dead bodies of land animals, human corpses, etc. Molisch made pure cultures of *Bacterium phosphoreum* upon a medium containing sodium chloride, peptone, and glycerine, and solidified with agar or gelatine (Fig. 166). By spreading the nutrient medium over the inner side of conical flasks of one or two litres capacity, he succeeded in making bacterial lamps (Fig. 167,

[1] *Vide infra*, p. 416.

[2] Hans Molisch, *Leuchtende Pflanzen*, Jena, 1904, p. 84.

left) which gave out a soft and perfectly steady blue-green light that was strong for about 14 days and weaker thereafter. With these lamps he was able to tell the time by his watch, observe the scale of a thermometer, read large print on the pages of books, recognise the face of a person 1–2 metres distant, and make photographs of statuettes (Fig. 167, right), thermometers, and other

Fig. 166.—A photograph of some luminescent colonies of *Bacterium phosphoreum*, made with their own light. The colonies, 6 days old, are growing in nutrient gelatine in a Petri dish covered with a glass plate. Time of exposure, 15 hours. Photographed by Hans Molisch. From his *Leuchtende Pflanzen*, Taf. I, Fig. 2.

objects. Molisch has suggested that bacterial lamps, on account of their cheapness, long unbroken luminosity, lack of smell, and absence of danger, might find some use—like certain luminous beetles in tropical lands—as night-lights.[1]

To obtain luminous bacteria on meat Molisch [2] advises that one should proceed as follows. Procure on successive days a few small pieces of meat (beef, veal) from a butcher's shop. Put them in a crystallising dish and pour over them a little 3 per cent. sodium

[1] Hans Molisch, *Leuchtende Pflanzen*, Jena, 1904, p. 124. [2] *Ibid.*, pp. 57–58.

chloride solution in such a manner that their bases become submerged. Set the dish in a cool room (about 10° C.), and cover

Fig. 167.—On the left, a bacterial lamp photographed by its own light. Colonies of *Bacterium phosphoreum* are growing in a layer of salt-peptone-gelatine which covers the side of the conical flask (1–2 litres capacity). The cotton-wool stopper in the mouth of the flask gives off no light whatever. Time of exposure, 12 hours. This lamp gave out a strong light for 14 days, and with it one could read a watch or the scale of a thermometer, recognise a person two metres away, etc. Reduced in size. On the right, a white porcelain bust of Schiller photographed by the light of nine bacterial lamps each of 1 litre capacity. Time of exposure, 15 hours. Reduced in size. Both photographs taken by Hans Molisch. From his *Leuchtende Pflanzen*, Taf. I, Figs. 1 and 5.

with a bell-jar. At the end of 1–4 days colonies of luminescent bacteria develop on the exposed surfaces of the meat and make the meat luminous in spots or patches. With Molisch 68 per cent. of the pieces of meat became luminous. At Winnipeg, some years ago, using the method just described, I found that about 50 per cent. of the pieces of meat became luminous.

It is noteworthy that in the Vegetable Kingdom the phenomenon of bioluminescence is never exhibited by any of the green plants—Algae, Bryophyta, Pteridophyta, and Phanerogamia—but is limited to the brown Peridinieae and to Bacteria and Fungi, non-chlorophyllous plants, which are either saprophytes or parasites and carry on their metabolism in a manner approaching that of animals. Bacteria and Fungi, as is well known, cannot perform the function of photosynthesis but obtain their energy, as do animals, from complex organic compounds. Furthermore, Fungi, in the production of such substances as chitin, glycogen, urea, sarkin, cetylalcohol, and cerebrosin, and in the non-production of chlorophyll, starch, and cellulose, show distinct chemical resemblances to animals.[1] It seems probable that the photogen contained in luminescent fungi is essentially the same as that of luminescent animals. Should future investigation prove that this supposition is correct, one further chemical resemblance between fungi and animals will have been established.

The best known forms of light-producing animals are : the dino-flagellates ; *Noctiluca* ; hydroids ; jelly-fish ; ctenophores ; sea pens ; *Chaetopterus* and other marine worms ; earth-worms ; brittle stars ; various crustaceans ; myriapods ; fireflies and glow-worms, the larvae of fireflies ; the molluscs *Pholas dactylus* and *Phyllirrhoe bucephala* ; squids ; *Pyrosoma*, a colonial ascidian ; and fishes.[2]

Luminous fungi and bacteria, as well as a few fish, give off light continuously and independently of stimulation. *Noctiluca* and most animals give off light only when they are stimulated.[3]

It is believed by Pierantoni (1918) and Paul Buchner (1921) that the luminescence of Fireflies, Cephalopods, and Ascidians

[1] For the chemistry of the Higher Fungi *vide* J. Zellner, *Chemie der Höheren Pilze*, Leipzig, 1907. Chitin occurs in the cell membranes of most fungi. Glycogen is found in Yeast cells, young asci, young basidia, etc. Urea has been obtained from *Lycoperdon Bovista* and *L. gemmatum* (Zellner, pp. 53–54). Sarkin has been obtained from *Fuligo septica* (Zellner, pp. 54, 235). Cetylalcohol occurs in *Fomes officinalis* (Zellner, pp. 20, 21, 39). Cerebrosin, which occurs in the brain and spinal column of animals, is believed to have been extracted from *Lycoperdon Bovista* (Zellner, p. 193).

[2] E. N. Harvey, *The Nature of Animal Light*, Philadelphia and London, 1920, p. 10.

[3] *Ibid.*, p. 69.

(*Pyrosoma*) is due to luminous symbiotic bacteria which reside in the luminous organs and pass from one generation to another *via* the eggs ; and it is suggested that the property of bioluminescence, in reality, is not possessed by any animals but is restricted to Fungi and Bacteria.[1] This interesting theory of symbiosis, of which doubtless we shall hear more in the future, is, as yet, far from being proved. E. N. Harvey,[2] in 1920, rejected its application to *Cypridina*, an ostracod Crustacean, because the luminous material of the animal will withstand boiling with 20 per cent. (by weight) hydrochloric acid for 6 hours. However, in 1921, the same investigator [3] found bacteria in the very large luminous organs of two fishes, *Photoblepharon* and *Anomalops*, which he examined at Banda Neira in the Dutch East Indies. The bacteria were always present in the organs, and emulsions of the organs glowed brightly in the dark and thus behaved exactly like emulsions of luminous bacteria. Luciferin and luciferase could not be demonstrated as being present and this, again, is characteristic of luminous bacteria. Moreover, the light emitted by the bacteria in the organs was, like the light of fungi and luminous bacteria generally, continuous both day and night, and unaltered by stimulation. Some of the bacteria, which were rod-shaped, when immersed in sea-water, were seen to move of their own accord, often with a corkscrew-like motion. Good growths of bacteria taken from the luminous organs were obtained on peptone-agar, but unfortunately they produced no light. Thus an important link in the chain of evidence required to prove that bacteria are responsible for the light emitted by the two fishes is still lacking.

The conditions for the emission of light by light-producing animals and plants include the presence of *oxygen*, *water*, and some *photogenic substance*.[4] Boyle, in 1667, proved the necessity of air for the luminescence of rotten wood and fish ; and Spallanzani, in 1794, showed that parts of luminous medusae gave no light when dried but, if moistened again, would emit light as before.[5] That lumines-

[1] *Vide* Paul Buchner, *Tier und Pflanze in intrazellularer Symbiose*, Berlin, 1921, chap. v, Die Leuchtsymbiose, pp. 340–400.

[2] E. N. Harvey, *The Nature of Animal Light*, 1920, pp. 14, 74.

[3] E. N. Harvey, *The Production of Light by the fishes Photoblepharon and Anomalops*, Publication No. 312 of the Carnegie Institution of Washington, pp. 43–60.

[4] E. N. Harvey, *The Nature of Animal Light*, 1920, p. 85.

[5] *Ibid.*

cent fruit-bodies of fungi do not give out light without the presence of oxygen is shown by the experiments of Kawamura described above ; and that they do not give out light without the presence of water is shown by my observation that fruit-bodies of *Panus stypticus* cease to give out light when dried, and resume their emission of light when again moistened.

Pflüger, in 1875, looked upon luminescence simply as a sign of intense respiration ; and Beijerinck, in 1915, regarded the light as an accompaniment of the formation of living matter from peptone.[1] Molisch (1904) and others, however, held the view that light-giving organisms contain a definite substance which undergoes oxidation and thus causes the emission of light. Molisch called this supposed substance *photogen*.[2]

In 1885–1887, Dubois made some important experiments on the mollusc *Pholas dactylus*. He found (1) that a hot-water extract of the luminous tissue emits no light, (2) that a cold water extract emits light for a certain time, and (3) that, if a hot-water extract and a cold-water extract no longer producing light are mixed together, they again produce light. Dubois, in 1887, therefore "advanced the theory that in the hot-water extract there is a substance *luciferin*, not destroyed by heating, which oxidises with light production in the presence of an enzyme *luciferase*, which is destroyed by heating. The luciferase is present together with luciferin in the cold-water extract, but the luciferin is soon oxidised and luciferase alone remains. Mixing a solution of luciferin and luciferase always results in light production until the luciferin is again oxidised."[3] Harvey found similar substances in some American fireflies (*Photinus* and *Photuris*), a Japanese firefly (*Luciola*), and in an ostracod crustacean, *Cypridina hilgendorfii*, and Crozier found them in *Ptychodera*, a balanoglossid. However, Harvey could not demonstrate their existence in luminous bacteria, in the annelid *Chaetopterus*, in certain pennatulids, in a squid, and in a fish ; but he points out that there are several reasons why the existence of luciferin and luciferase might be difficult to demonstrate.[4] It may

[1] E. N. Harvey, *The Nature of Animal Light*, 1920, p. 102.
[2] H. Molisch, *loc. cit.*, p. 109.
[3] E. N. Harvey, *loc. cit.*, p. 103.
[4] *Ibid.*

well be that it is only owing to some difficulty in extraction that at present these substances have not been shown to exist in any fungus. Ewart [1] using *Pleurotus candescens*, Kawamura [2] using *Pleurotus japonicus*, and I myself using *Panus stypt. luminescens*, have each found that the juice extracted from a luminous fruit-body is non-luminous.

For luminous organisms in general, including *Panus stypt. luminescens*, *Pleurotus japonicus*, and other luminous fungi, it appears from the facts already brought forward that Molisch was right in postulating a photogen, and that the photogen has a dual nature in that it consists of luciferin, which is thermostable, and of the enzyme luciferase, which is thermolabile. The interaction of analogous pairs of thermostable and thermolabile substances is required in some other biological processes, *e.g.* the alcoholic fermentation of glucose by the zymase of Yeast juice, where a thermostable co-enzyme and the enzyme proper, which is thermolabile, are both required for fermentation to be carried on.[3]

When luciferin in oxidised, it is converted into *oxyluciferin*. Under the influence of reducing agents oxyluciferin becomes reduced and the luciferin is regenerated. In this connection Harvey says: "We may quite logically believe that the bacteria and fungi oxidise luciferin in one part of the cell and reduce oxyluciferin in another, while the animal organisms oxidise luciferin during the flash and reduce it between flashes. Should this be true, and it is not proven as yet, living processes would present a marvel of economy quite at variance with the prodigality we are apt to associate with reproductive nature." [4]

Luciferase, according to Harvey,[5] "is unquestionably a protein and all its properties agree with those of the albumins. Although used up in oxidising large quantities of luciferin, it behaves in many ways like an enzyme and may be so regarded." Luciferin,

[1] A. J. Ewart, "Note on the Phosphorescence of *Agaricus* (*Pleurotus*) *candescens* Müll." *The Victorian Naturalist*, vol. xxiii, 1906, p. 174.

[2] S. Kawamura, *loc. cit.*, p. 9.

[3] E. N. Harvey, *The Nature of Animal Light*, 1920, p. 104.

[4] E. N. Harvey, "Animal Luminescence," *Journal of the Franklin Institute*, July, 1923, pp. 38, 41.

[5] E. N. Harvey, *The Nature of Animal Light*, 1920, pp. 141–142.

according to the same investigator,[1] "has many properties in common with the proteoses and peptones, but its chemical nature cannot be definitely stated at present."

From experiments made upon extracts of the ostracod crustacean *Cypridina*, Harvey [2] concludes that it is safe to say: (1) that one part of pure luciferin in between 4,000,000,000 and 40,000,000,000 parts of sea water gives, upon oxidation in the presence of luciferase, light which is visible to the naked eye, and (2) that one part of pure luciferase in between 800,000,000 and 8,000,000,000 parts of sea water will oxidise a stock luciferin solution with visible luminescence. It thus appears that the eye can detect the luminescence of extraordinarily weak solutions of luciferin and luciferase. Indeed, as Harvey points out, the concentrations of luciferin and luciferase which can be detected by the sight test are far smaller than those of other substances required for detection by ordinary chemical reactions.[3]

The light given out by luminous animals may not be always functionally useless. It is possible that certain deep-sea fish, as well as squids and shrimps, which have complex luminous organs, use them as lamps. The light organs of *Photoblepharon* and *Anomalops*, the two surface fishes recently investigated by Harvey, are believed to be used as search-lights to attract prey.[4] The light given out by fireflies, by the toad fish, *Porichthys*, which is only luminous in the spawning season, and by the worm *Odontosyllis* of Bermuda, which is brilliantly luminous when swarming, may serve as a means of recognition or as a sex signal to bring together the sexes for mating.[5] It has always been suggested that the light of the sea-pens serves to scare away predaceous animals.[6] In many animals, however, the emission of light appears to be quite useless. Useless, too, we must consider the emission of light by luminescent bacteria and by

[1] E. N. Harvey, *The Nature of Animal Light*, 1920, p. 142.

[2] E. N. Harvey, "The Minimum Concentration of Luciferin to give a Visible Luminescence," *Science*, 1923, pp. 501–503.

[3] It is remarkable that the eye can detect the flash of light that is given out by the impact of a single charged helium atom on fluorescent zinc sulphide, but here the light has a mechanical origin, not a chemical.

[4] E. N. Harvey, *The Production of Light by the Fishes Photoblepharon and Anomalops*, Publication No. 312 of the Carnegie Institution of Washington, p. 48.

[5] E. N. Harvey, *The Nature of Animal Light*, 1920, p. 83.

[6] *Ibid.*

the mycelium of *Armillaria mellea* growing within a rotten log or in the stump of a tree. I fail to see any biological advantage in the emission of light from the fruit-bodies of luminous species of Pleurotus and Panus. Ewart,[1] in writing about *Pleurotus candescens*, says: "As to the biological significance of the luminosity, its restriction to the gills shows that its present function is evidently to attract molluscs, as well as creeping and flying insects, which may aid in the distribution of the spores." With this view I cannot agree. There is no evidence that the light emitted by the fungus does attract the animals mentioned. The light would be useless for such a purpose during the day. Slugs have very weak eyes and a very strong olfactory sense. As I have shown in Volume II, they find distant fungi chemotactically.[2] It is not certain that they could perceive a luminous fungus in the dark unless they were very near to it, and it is very doubtful whether they would crawl towards an object, at night in the open, merely because it emitted light. The phototaxis hypothesis therefore seems to me to be highly improbable. I am rather of the opinion that the emission of light by fungus fruit-bodies is of no biological advantage whatever, but takes place merely as an accompaniment of some chemical reaction necessitated by metabolism.

Further Observations on the Bioluminescence of Panus stypticus luminescens.—Stimulated by reading Kawamura's paper on *Pleurotus japonicus* published in 1915 and Harvey's book entitled *The Nature of Animal Light* published in 1920, I reinvestigated the bioluminescence of *Panus stypt. luminescens* in the autumn of 1921. The results of this second investigation will now be communicated.

Panus stypt. luminescens occurs but very rarely in central Canada. It was therefore necessary for me to procure fruit-bodies from other parts of North America. Material was kindly supplied to me from Toronto by Professor J. H. Faull, from Ann Arbor, Michigan, by Professor C. H. Kauffman, and from Minneapolis by Professor E. C. Stakman. It was sent in the dry condition and, after arrival, it was revived as required by moistening it. The younger fruit-bodies in all the collections proved to be luminescent.

[1] A. J. Ewart, *loc. cit.*, p. 174.

[2] These *Researches*, vol. ii, 1922, pp. 222–235.

The fruit-bodies used for my observations were usually removed from the woody substratum upon which they grew, and wet cotton wool was applied to their upper surfaces for an hour or two until they had absorbed all the water that they could take in. They were then placed, gills downward, on wet cotton wool covering the base of a large crystallising dish, and the dish was closed with a glass plate. Under these conditions many of the fruit-bodies continued to glow for 7–10 days. The examination of the fruit-bodies for luminescence took place in a photographic dark-room kept at a temperature of about 18°–22° C.

To observe the light given out by a fruit-body it is necessary that one's eyes should be in the proper physiological condition. I found that, if I went into the dark-room at mid-day after my eyes had been acted upon by bright daylight, I was often obliged to wait 5 minutes or even longer before I could see any glow at all, even from the brightest fruit-bodies. Subjection to darkness enormously increases the sensitiveness of the eyes; and, after I had spent 10 to 15 minutes in the dark-room, the fruit-bodies usually appeared to glow with their maximum intensity. Experience, supported by a series of exact experiments already recorded,[1] has taught me that my eyes are much more sensitive to feeble light in the evenings after sunset than at mid-day. After an hour's walk in the evening in the dark, or after sleep, I found that I could see the light of the fungi immediately I entered the dark-room, and could even perceive it distinctly outside the room in weak diffuse daylight. That Dr. Bisby, one evening when walking in a wood, discovered a group of the fruit-bodies by the light they gave out has already been mentioned.[2] At the time, his eyes must have been in a highly sensitive condition. In studying the luminescence of fungi, there can be no doubt that the physiological condition of the eyes is a factor of considerable importance.

In full-grown fruit-bodies the under surface of the pileus is strongly luminous, the upper side faintly luminous, and the stipe not luminous at all. The gills are principally responsible for the emission of the light, but there is a certain amount of radiation emitted also by the pileus-flesh. From a luminous fruit-body

[1] *Vide supra*, pp. 373-376.

[2] *Vide supra*, p. 376.

I scraped away the gills with a sharp penknife, and the pileus-flesh still continued to glow, although quite feebly.

When the fruit-body begins its development as a tiny rudiment on the exterior of a block of wood, it is brightly luminous all over its surface. As differentiation proceeds, light is emitted not merely by the rudimentary pileus but also by the enlarging stipe. As soon as the stipe has become full-grown, it ceases to give out light, and then only the developing pileus is luminous. For some time the pileus gives out light from both its surfaces, although more strongly from its under surface. Then the luminescence becomes concentrated in the gills alone, where the production and liberation of spores is still in active progress; and, finally, as the hymenium becomes more and more exhausted, even the light emitted by the gills grows dim and disappears. It therefore seems that the intensity of the light given out by any part of a fruit-body is directly proportional to the rate of development. The emission of light by the hyphae of *Panus stypt. luminescens*, like the emission of heat during respiration, is (as we shall see) an oxidative process: and, like the emission of heat during respiration, it appears to increase in intensity with an increase in the rapidity of growth, and *vice versa*.

The colour of the light emitted by *Panus stypt. luminescens* has always appeared to me to be greenish. There is certainly no red in it. When working in the dark-room I found that, after using a red light and then switching it off, I could at once perceive the light of the fungus, whereas after using a white light perception only took place with some delay. The more favourable result with the red light seems to have been due to the fact that a red light does not tire one's eyes for seeing a green light.

When one's eyes are in a sensitive condition, it is possible to perceive the light given out by the under side of a group of fruit-bodies at a distance of several feet. When about six fruit-bodies were set together in a dark laboratory, I could clearly see the light which they gave out at a distance of 10 feet and just perceive the light at a distance of even 30 feet.

A fruit-body is most strongly luminous when it is just full-grown. Thereafter, as it exhausts itself by shedding spores its light gradually

declines, finally becoming invisible shortly before the end of the spore-discharge period. The longest period of luminescence for any one fruit-body, severed from its substratum and kept on wet cotton wool in the laboratory, was about ten days.

A fruit-body takes about 10 days to develop from a tiny rudiment to full size and is luminescent from its first appearance on the surface of the wood. Since mature fruit-bodies when taken into the laboratory may give out light for 7 to 10 days, doubtless under natural conditions the duration of the bioluminescent period is at least three or four weeks. Some fruit-bodies which developed on a block of wood in the laboratory gave out light for about a month; and one such fruit-body, which grew under deficient moisture conditions, was actually luminescent for three months.[1]

In the hope of extracting the photogen I placed a highly luminescent fruit-body between two glass plates and pressed the plates tightly together with my fingers. Within a minute the fruit-body had been completely flattened, its sap expressed, and its cells broken up. As the crushing process began, the light began to fade, and when it was completed, the light was no longer emitted. The sap extracted, therefore, was non-luminous.

A dried fruit-body was taken and broken into powder with the help of a pestle and mortar. The powder was then taken into the dark-room and moistened with water. The mixture of powder and water never emitted any light at all.

The two experiments just described indicate that it is not possible to extract a photogen from *Panus stypt. luminescens* by mechanically squeezing out its sap. This is in accord with the experience of other workers on luminescent fungi. As already mentioned,[2] Kawamura failed in an attempt to extract a photogen from *Pleurotus japonicus* and Ewart failed in a similar attempt with *Pleurotus candescens*.

The difficulty in extracting a photogen from fungi is doubtless due in part to the fact that the photogen in these plants is not present as an extracellular secretion or excretion, as it is in some animals such as *Pholas*, but is formed intracellularly.[3] When the hyphae

[1] *Vide infra*, p. 395.
[2] *Vide supra*, p. 382.
[3] *Cf.* H. Molisch, *loc. cit.*, p. 118.

containing the photogen are killed, great chemical confusion must result. Various substances kept isolated from one another in the living protoplasm rush together, and doubtless some of the numerous chemical reactions and physical changes that go on are fatal to the photogen.

The spores are non-luminous. I examined dense fresh spore-deposits which had been moistened with water or an agar nutrient solution, but could not perceive that any light ever came from them. This is in accord with the experience of Tulasne[1] with dense spore-deposits of *Pleurotus olearius* and of Kawamura[2] with the spores of *Pleurotus japonicus*.

It is worthy of note that, whereas the spores of luminous fungi are non-luminous, the eggs of certain luminous animals, *e.g.* Glow-worms (Lampyris), emit light whilst they are in the ovary and after being laid.[3] According to the theory of symbiosis already mentioned,[4] the luminescence of these eggs is due to their containing luminous bacteria.

Photographs made with the Light of the Gills.—To obtain photographs made with the light of *Panus stypt. luminescens* I proceeded as follows, my method being identical with that used by Kawamura for making photographs with the light of *Pleurotus japonicus*. In the dark-room a rapid photographic quarter-plate[5] was laid on a table with the film turned upwards. The plate was then covered with a thin sheet of plain glass to the under surface of which five black paper letters, making the word PANUS, had

[1] L. R. Tulasne, "Sur la phosphorescence spontanée de l'*Agaricus olearius* DC., etc.," *Ann. d. sci. nat.*, sér. 3, T. IX, 1848, pp. 338–364.

[2] S. Kawamura, *loc. cit.*, pp. 8–9.

[3] *Cf.* E. N. Harvey, *loc. cit.*, p. 11. F. H. Fabre in his book *The Wonders of Instinct* (London, 1918, pp. 287–288) thus characteristically describes the bioluminescence of the eggs of the Glowworm: "Here is a very singular thing; the Glowworm's eggs are luminous even when they are still contained within the mother's womb. If I happen by accident to crush a female big with germs that have reached maturity, a shiny streak runs along my fingers, as though I had broken some vessel filled with a phosphorescent fluid. The lens shows me that I am wrong. The luminosity comes from the cluster of eggs forced out of the ovary. Besides, as laying time approaches, the phosphorescence of the eggs is already made manifest. . . . A soft opalescent light shines through the integument of the belly."

[4] P. 380.

[5] Two kinds of plates were used: (1) Royal, special extra rapid, speed 180 Watkins and (2) Seed, R., speed 280 H.D.

been stuck with gum. The black letters, which were each 0·8 inch high and collectively 2 inches wide, therefore pressed directly upon the film of the photographic plate beneath. Upon the top of the sheet of plain glass were then set about twenty *Panus stypt. luminescens* fruit-bodies with their strongly luminous under surfaces looking directly downwards toward the letters and the film. To prevent

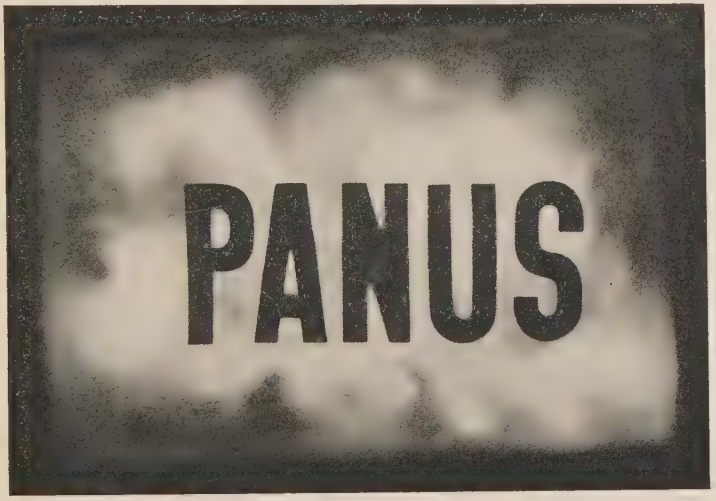


Fig. 168.—A shadow-photograph of the black-paper letters of the word *Panus* made with the light of about twenty small fruit-bodies of *Panus stypticus* p.f. *luminescens* gathered at Saint Paul, Minn., U.S.A. The rectangular form of the lighted portion was due to an inverted cardboard box which covered the fruit-bodies. Exposure 15 hours. Reproduced the original size.

the fruit-bodies from drying up, they were covered over with a small, inverted, lidless, cardboard box which was weighted above to keep it in place. A good photograph of the letters was obtained with an exposure of two hours, but the one shown in Fig. 168 was made with an all-night exposure of fifteen hours. While the photograph was being taken, the spores liberated by the twenty fruit-bodies formed as many thick white spore-deposits upon the sheet of glass.

By similar means to those just described was made the photograph of the black paper star shown in Figure 169. On this occasion about twelve fruit-bodies were employed, the fruit-bodies were covered with an inverted crystallising dish, and the exposure was

for eleven hours. In other experiments smaller photographs were made with four fruit-bodies only ; and, no doubt, if one so desired, very small photographs could be made with the light of a single fruit-body. Exposure of the plates for two hours gave good results, but shorter exposures than this were not attempted.

The Emission of Light by the Mycelium.—Some of the Panus fruit-bodies sent to me by my North American correspondents

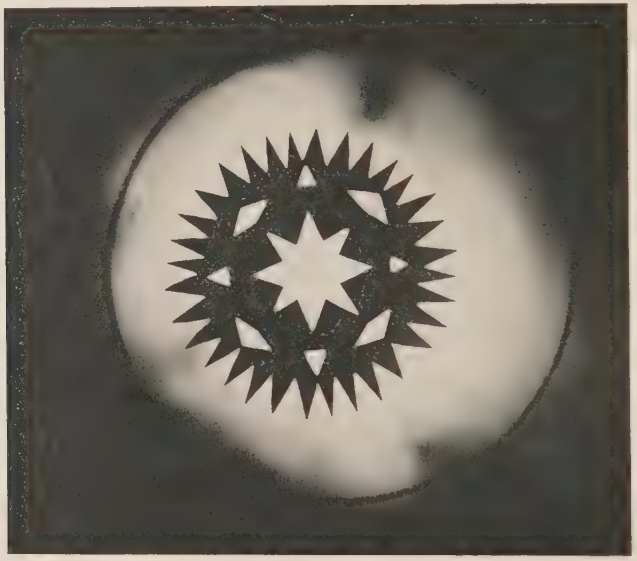


Fig. 169.—A shadow-photograph of a black-paper star made with the light of about twelve fruit-bodies of *Panus stypticus* p. f. *luminescens* gathered at Saint Paul, Minn., U.S.A. The black circle at the periphery was caused by the base of an inverted crystallising dish. Exposure 11 hours. Reproduced the original size.

were attached to pieces of wood which had been broken away from stumps, logs, etc. This material was moistened by placing it on wet cotton wool. After some hours or days I observed that not only did the fruit-bodies emit light but, in some instances, also the mycelium at the broken surface of the wood. Sometimes, however, pieces of wood bearing fruit-bodies never showed the least sign of luminescence. To settle the question of the luminosity of the mycelium I therefore resorted to the method of pure cultures. I placed a fruit-body on a sterilised glass slide and soon obtained

a dense spore-deposit. Some of the spores were then sown on dung-agar poured into Petri dishes. After a few days they germinated. The mycelia grew slowly as if in an unsuitable medium. When they had attained a diameter of 1–2 mm., I examined them night after night in the dark-room, but could not detect any light coming from them. In the belief that this might be due to the small amount of mycelium present or its lack of vigour, I therefore made some cultures upon wood. Some blocks of Silver Birch wood, 3 inches long, 2 inches wide, and octagonal in cross-section, were boiled in water, then taken out of the boiling water and plunged in cold water, this process being repeated several times until the blocks were waterlogged. Then the blocks were placed in large glass jars provided with cotton-wool plugs (Fig. 170) and were

FIG. 170.—Culture of the luminous mycelium of *Panus stypticus* physiological form *luminescens*. Spores obtained from fruit-bodies gathered at Saint Paul, Minn., U.S.A., were sown on the top of the sterilised block of Birch wood which rests on wet cotton wool. The mycelium is now spreading over the block. Photographed by daylight. About $\frac{3}{5}$ the actual size.

sterilised by subjecting them in an autoclave to 15 pounds steam pressure for half an hour. One of the blocks was then inoculated with spores taken from a spore-deposit, and a second block was inoculated with a piece of agar containing mycelium taken from one of the Petri-dish cultures already mentioned.[1]

The spores sown on the first of the two blocks soon germinated and produced a mycelium. When the mycelium was very small I examined it in the dark-room, but could not perceive any light coming from it. However, when it had attained a diameter of about 3 cm. (Fig. 170), I re-examined it one evening when my eyes were well rested and very sensitive. To my surprise I at once perceived that it was distinctly luminous. There was a zone of bright light, about 6 mm. wide, at the periphery of the mycelium where the hyphae had a woolly appearance and were spreading over the substratum, while the central part of the mycelial disc appeared relatively dark and only feebly luminous. In the course of the next six weeks the mycelium completely covered the block of wood and even grew downwards over and into the cotton wool below. The whole block and the cotton wool thus became luminous and I was able to assure myself that, although the peripheral rapidly growing hyphae were giving out the brightest light, yet even the oldest part of the mycelium was giving out a certain amount of light also. In the dark-room I could readily observe the shape of the block, and I could perceive its light at a distance of 10 or even 20 feet. The colour of the light appeared to me to be greenish-white like that of the fruit-bodies. A comparison of the mycelium on the wood with the under side of a vigorous fruit-body, made in the dark-room, proved that for equal areas the light emitted from the mycelium is distinctly less intense than that emitted from a fruit-body. I suspect that even the youngest mycelia are luminescent and that my failure to detect the phenomenon in very young mycelia was simply due to the feebleness of the light emitted. Possibly this view might be substantiated by means of photography.

The mycelium on the blocks of wood in the cultures continued to give out light for upwards of a year; and, during this period, it

[1] For undertaking the work of preparing the culture media and making the inoculations I am indebted to Miss Irene Mounce.

gave rise to numerous, small, somewhat abortive but luminous, *Panus stypt. luminescens* fruit-bodies.

The above observations clearly prove that, in *Panus stypt. luminescens*, not only is the fruit-body luminescent but also the mycelium. This emission of light in both the vegetative and the reproductive stages of development is at present known to occur in only one other luminous Hymenomycete, namely, *Clitocybe illudens*.[1] Kawamura[2] states that, in *Pleurotus japonicus*, the gills alone are luminous and that the pileus-flesh and the mycelium are non-luminous. On the other hand in *Armillaria mellea*, as is well known, the mycelium vegetating in the wood, the rhizomorpha subcorticalis, and the developing rhizomorpha subterranea are luminous ; but the fruit-body is non-luminous.

A Photograph made with the Light of the Mycelium.—A shadow-photograph of a dried frond of a fern was made with the light given out by the mycelium of *Panus stypt. luminescens* in the following manner. The fern leaf was held in a printing frame against the film of a photographic plate by means of a sheet of clear glass, and then the frame was set as close as possible to the jar containing the luminous mycelium.[3] After the plate had been exposed to the light of the mycelium for 24 hours, it was developed ; and the negative yielded the print which is reproduced in Fig. 171. A study of the photograph shows that the light of the mycelium was sufficiently strong to penetrate somewhat the whole thickness of the leaflets except where these bore their black sori.

Dependence of Luminescence on the Presence of Moisture.—There can be no doubt that in fungi, as in animals, the emission of light is dependent on the presence of moisture. In support of this statement clear evidence can easily be obtained by making experiments with *Panus stypt. luminescens*. I found that dry

[1] W. A. Murril ("Luminescence in the Fungi," *Mycologia*, vol. vii, 1915, p. 132) observed that the wood on which some fruit-bodies of *C. illudens* grew was luminous in the dark and therefore concluded (p. 115) that the mycelium of *C. illudens* is luminous. There is, however, the bare possibility that the mycelium in the wood may have belonged to some other fungus, *e.g. Armillaria mellea*. It is therefore desirable that some one should establish the luminosity of the mycelium of *C. illudens* on the basis of pure cultures.

[2] S. Kawamura, *loc. cit.*

[3] The luminous mycelium had grown about to the extent shown in Fig. 175, p. 412.

fruit-bodies, when examined in the dark-room, give out no light at all, but that after dry fruit-bodies are moistened by holding them

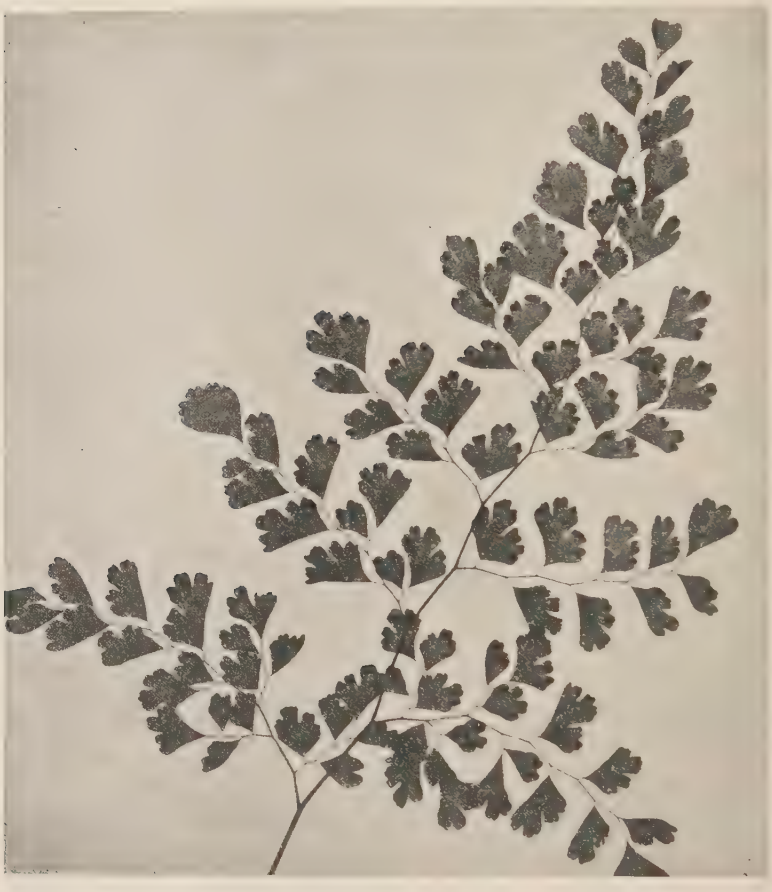


Fig. 171.—A shadow-photograph of a dried frond of Adiantum made with the light given out by the mycelium of *Panus stypticus* physiological form *luminescens* of North American origin. The luminescent mycelium was growing down the side of a block of Birch wood contained in a fruit-jar (*cf.* Fig. 170). The fern leaf was held in a printing frame against the film of a photographic plate, and the frame was set as close to the fruit-jar as possible. Plate developed after 24 hours exposure. Natural size.

at intervals in a stream of water under a tap their luminescence is very soon regained.

On several occasions, when my eyes have been in a sensitive condition, I have wetted a dried fruit-body in the dark-room and

have then recorded the time which elapsed between the moment of wetting and the moment when I first perceived the emission of light from the gills. The following are the lengths of four of the recovery-periods thus measured: (1) 6 minutes, (2) 5 minutes, (3) 10 minutes, all for fruit-bodies kept dry for two to three months, and (4) 8 minutes for some fruit-bodies kept dry for six months.

When a luminescent fruit-body is allowed to dry up, it soon ceases to shed spores, and then its outer edges curl downwards and inwards as already described in an earlier Section. Light still continues to be emitted after spore-discharge has ceased and even when the curling-up process is far advanced. Comparatively little water therefore seems to be necessary to permit of the photogen being active. Light ceases to be emitted only after the fruit-body has lost most of its water and is becoming dry and tough. When a dried curled-up fruit-body is wetted, it begins to emit light almost before the uncurling process has begun.

A block of wood bearing *Panus stypt. luminescens* fruit-bodies was removed from a stump of a tree and set in a bottle like that shown in Fig. 170 (p. 391). After a few weeks some new fruit-bodies grew out from the block. Two of them developed quite normally and, as usual, were brightly luminous. Owing to deficient water-supply from the wet cotton wool beneath the block, one of the fruit-bodies curled downwards and inwards, dried up, and ceased to be luminous. The other one grew so that its upper surface came to be applied to the surface of the wood of the somewhat moist block. Under these conditions its pileus did not curl downwards and inwards, but remained flattened and continued to give out light from its under surface for three months. Now it had been observed that fruit-bodies severed from their substrata and placed on wet cotton wool in a damp-chamber give out light for only about 7–10 days. It was therefore surprising to find that the fruit-body just described, after becoming full-grown, continued to emit light for three whole months.[1] To account for this relatively very long luminescent period we may suppose that the fruit-body

[1] Actually 87 days. At the end of this period the fruit-body was still emitting light feebly, but owing to my departure from Winnipeg, I could make no further records.

contained too little water to enable its hymenium to undergo rapid development and exhaustion, and yet enough water to permit of the continuance of the oxidative process which results in the emission of light. Doubtless, if one were to take a partially dried fruit-body and keep it in a damp atmosphere so that it could not dry up, it would continue to give out light far longer than another similar fruit-body which was fully saturated with water.

Dependence on Oxygen.—Luminous fungi, like all other luminous organisms, only emit light in the presence of oxygen. This was first proved by Robert Boyle,[1] who experimented on the effect of removing the air from a piece of luminous wood. It is probable that the wood contained the mycelium of *Armillaria mellea*, for in England this fungus, which has a luminous mycelium, is very commonly found upon stumps and at the base of dead trees. Boyle on October 29, 1667, thus described his experiments :

"Exp. I.: Having procured a Piece of *shining Wood*, about the bigness of a groat or less, that gave a vivid light (for *rotten Wood*), we put it into a middle sized *Receiver*, so as it was kept from touching the Cement ; and the *Pump* being set a-work, we observed not, during the 5 or 6 first Exsuctions of the Air, that the Splendor of the included Wood was manifestly lessened (though it was never at all increased ;) but about the 7th Suck, it seemed to glow a little more dim, and afterwards answered our Expectation, by losing of its Light more and more, as the Air was still farther pumped out ; till at length about the 10th Exsuction, (though by the removal of the Candles out of the Room, and by black Cloaths and Hats we made the place as dark as we could, yet) we could not perceive any Light at all to proceed from the *Wood*.

"Exp. II. : Wherefore we let in the outward Air by Degrees and had the pleasure to see the seemingly extinguished Light revive so fast and perfectly, that it looked to us almost like a little Flash of Lightning, and the Splendor of the Wood seemed rather greater than at all less, than before it was put into the Receiver."

Kawamura[2] observed that, when a fruit-body of *Pleurotus*

[1] Robert Boyle, *Phil. Trans. Roy. Soc.*, Abridged, 5th Ed., vol. ii, 1722, p. 206; and vol. iii, 1749, p. 646.

[2] S. Kawamura, *loc. cit.*, pp. 19–21.

japonicus was plunged into a jar containing either hydrogen or pure nitrogen gas, the emission of light ceased in the course of a few minutes. He also found that the fruit-body recovered its luminescence when it was again given access to the oxygen of the air.

For investigating the effect produced upon the luminescence of *Panus stypt. luminescens* by replacing the air surrounding a fruit-body by hydrogen I made use of the apparatus shown in Fig. 172. The hydrogen was generated by the action of hydrochloric acid upon zinc in the Kipp apparatus *a*; and the passage of the gas through the wash-bottle *c* and through the experimental tube *e* was controlled by the stop-cock *b*. In order to prepare for an experiment, the tap *b* was opened and hydrogen was allowed to flow through the wash-bottle (containing water) and the experimental tube until all the air had been completely driven out of the apparatus. The tap *d* was then closed. Next, the rubber stopper *h* was pulled out from the end of the experimental tube so as to allow the hydrogen in the tube to escape and be replaced by air. Then two luminous fruit-bodies *g* were inserted into the end of the tube, as shown in the illustration, and the stopper *h* was then put back into place. The electric light of the dark-room in which the apparatus was set up was now switched off. I waited for a few minutes until the sensitiveness of my eyes to light had greatly increased and I could clearly see the glow coming from the under surfaces of the two fruit-bodies. Then the tap *d* was turned on, thus allowing the hydrogen gas to rush through the experimental tube *e* and drive out the air which it contained.

Immediately after the hydrogen gas was allowed to flow through the tube *e*, the light of the fruit-bodies began to grow dim, and within three seconds of turning on the tap *d* every trace of luminescence had vanished. Shortly after the fruit-bodies had ceased to emit light, the taps *i* and *d* were closed in succession. The hydrogen surrounding the fruit-bodies thus came to be imprisoned in the tube *e*. So long as the tube contained hydrogen, the fruit-bodies never emitted any light whatever. After the fruit-bodies had remained in the hydrogen for 5 minutes, trial was made of the effect of suddenly giving them access to the oxygen of the air.

This was accomplished by pulling out the stopper *h* from the end of the tube. Immediately the stopper was pulled out of the tube, the fruit-bodies began to emit light and, at the end of only

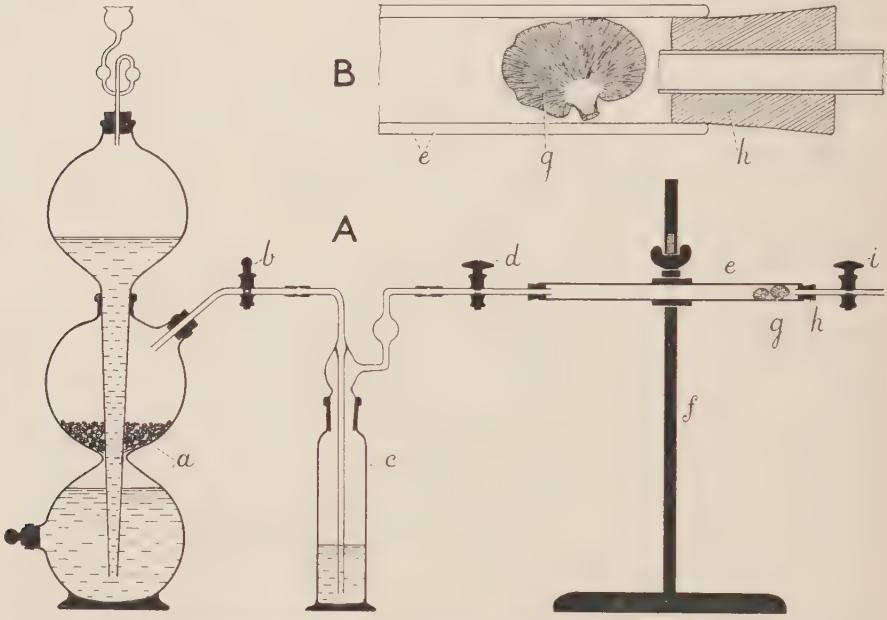


FIG. 172.—A, Apparatus for demonstrating that the luminescence of *Panus stypticus* physiological form *luminescens* is dependent upon the presence of oxygen: *a*, a Kipp apparatus for generating hydrogen; *c*, a wash-bottle containing water; *e*, the experimental tube containing two Panus fruit-bodies at *g*, held by the stand *f*; *b*, *d*, and *i*, stop-cocks for controlling the passage of the hydrogen through the apparatus; *h*, a rubber cork that can be readily pulled out of the end of the tube *e*. B, a detail of the experimental tube, actual size. By driving out the air in the experimental tube *e* with hydrogen, the light given out by the fruit-bodies is extinguished within three seconds. If the stop-cocks *d* and *i* are closed and the tube *e* is full of hydrogen, and if then the cork *h* be pulled out so that air rushes into the tube *e*, the fruit-bodies begin to give light again within one second.

about one second, they were emitting light with their maximum intensity.

The experiment just recorded, with slight variations in the length of time the fruit-bodies were allowed to remain in the hydrogen, was repeated again and again; and it was always found that the light of the fruit-bodies completely disappeared within three seconds of turning on the stream of hydrogen and reappeared within one second of pulling out the stopper *h* and allowing oxygen to

re-enter the end of the tube.[1] This perfect control of the fungus light, made possible by removing air from the fruit-bodies or restoring it to them, and actually effected by the operation of the taps *d* and *i* and the stopper *h*, finds an analogy in the control of electric light by turning a switch off and on.

The fact that one can turn the light of the fruit-bodies on or off practically instantaneously by supplying or withholding oxygen proves conclusively that in *Panus stypt. luminescens* the photogen is absolutely dependent for its light-giving power on the presence of oxygen. There can be no doubt, therefore, that here, as in other luminous organisms, the emission of light is an oxidative process. The oxidative process, however, as indicated by the investigations made upon it by animal biologists, is in all probability quite distinct from the oxidative process that is associated with respiration.[2]

A Kipp apparatus generating carbon dioxide was substituted for the Kipp apparatus generating hydrogen in the apparatus shown in Fig. 172, and a series of experiments like those just described was then made. When the air surrounding the luminous fruit-bodies was replaced by carbon dioxide, the light immediately grew dim, and it ceased to be emitted within about three seconds. When, by pulling out the stopper *h*, air was allowed to re-enter the experimental tube, the fruit-bodies immediately became luminous and within about one second regained their normal intensity of luminescence.

Some pure nitrogen was prepared by slowly bubbling air through a strong solution of ammonia and then passing it over copper turnings heated to redness. The nitrogen was collected by siphoning it into a two-litre bottle which was first filled with water acidulated with sulphuric acid. When about a litre of nitrogen had been collected, the intake pipe was carefully washed with dilute sulphuric acid to remove the last traces of ammonia. The nitrogen-gas bottle, together with the siphon and water-bottle, was then attached to

[1] Had the experimental tube *e* been much shorter, say 1·5 inches long instead of 7 inches, the hydrogen, when allowed to flow, would certainly have replaced the air in the tube much more rapidly; and then, no doubt, the disappearance of the fungus light would have taken place in much less than three seconds—perhaps in less than one second. This variation in the experiment, however, was not tried.

[2] *Vide*, E. N. Harvey, *loc. cit.*, pp. 99–100.

the wash-bottle and the experimental tube containing some luminous fruit-bodies, and the gas was forced through the tube by raising the water-bottle. The nitrogen gas affected the luminescence of the fruit-bodies in exactly the same manner as the hydrogen and the carbon dioxide : when it was caused to replace the air, the light of the fungi disappeared within three seconds ; and, when the air was allowed to replace the nitrogen the fungi became luminous immediately, and they glowed again with maximum intensity within about one second after obtaining access to the air.

Oxygen from an oxygen cylinder was caused to pass through the wash-bottle *c* and the experimental tube *e* containing the fruit-bodies, but no increase or decrease in the intensity of the light emitted could be observed.

It was found that diluting the air slightly with hydrogen or carbon dioxide did not appreciably affect the intensity of the luminescence of the fruit-bodies. Although this dilution was not studied quantitatively, I think that there can be no doubt that the percentage of oxygen per unit volume of ordinary air is much higher than is necessary to allow the emission of light to take place with its full intensity or, to state the matter in other words : the limiting factor for the intensity of light-emission from the fruit-bodies of *Panus stypt. luminescens* under natural conditions is not the percentage of oxygen in the air but in all probability the amount of one or other components of the photogen which, until we have evidence to the contrary, we may assume to be composed of luciferin and luciferase.

Effect of Anaesthetics.—A few experiments were made with ether and chloroform.

One cc. of ether was dropped into a 250 cc. measuring flask and a stopper inserted into the neck. A luminous fruit-body was then spitted upon a pin attached to another stopper. The stopper closing the flask was then replaced by the stopper bearing the fungus. The fruit-body, on thus becoming subjected to ether vapour, soon became less luminous. The light gradually diminished and, at the end of about 20 seconds, had disappeared. After the fruit-body had been kept in the ether flask for another 40 seconds, it was removed to the air of the room. Thereafter the light gradually returned : it

became visible at the end of about 20 seconds from the removal, and was fairly bright at the end of 60 seconds. The fruit-body was then placed on wet cotton wool in a damp-chamber. The next evening it was found to be glowing with its full intensity. This experiment, with slight variations, was repeated several times. It thus appears that, under certain conditions, ether vapour temporarily arrests the action of the photogen.

A luminous fruit-body which was kept in the ether flask for 18 hours was found at the end of this time to be non-luminous and to have lost its vitality. Long subjection to ether vapour therefore seems to be fraught with fatal consequences.

One cc. of chloroform was placed in the 250 cc. measuring flask instead of ether, and a glowing fruit-body was inserted as before. The light of the fungus rapidly diminished in intensity and, at the end of 15 seconds, had entirely vanished. After 5 more seconds the fungus was removed from the flask. Two seconds after this removal, the light of the gills began to return ; the glow then brightened but soon began to grow dim again ; and within one or two minutes of the removal became entirely invisible. This experiment was repeated with another fruit-body with the same result. These fruit-bodies were placed in a damp-chamber but they did not recover their luminosity, and next day they were found to be dead. It is evident therefore that 1 cc. of chloroform in a 250 cc. flask is much more fatal to a fruit-body than 1 cc. of ether.

Effect of Temperature.—The range of temperature required for the emission of light was determined by means of the following experiments.

0° C. To subject luminous fruit-bodies to a temperature of 0° C., an ice-bath was employed in the manner indicated by Fig. 173. Two luminous fruit-bodies were placed in a test-tube (*a*) containing a thermometer held in position by means of a folded rubber band and cotton wool. A large block of ice (a part only shown at *c*) was then taken into the laboratory and a deep cavity made in it. This cavity was filled with ice-water (*b*). The test-tube containing the fruit-bodies and thermometer was then pushed down into the ice-water and covered with an inverted beaker (*d*) lined with cotton wool. The block of ice and the beaker were then covered over

with sacking. The air in the test-tube surrounding the fruit-bodies soon attained a temperature of 0° C., as was proved by lifting the beaker and observing the thermometer.

The fruit-bodies in the test tube were subjected to a temperature of 0° C. for a period of 11 hours. At the end of this time, crushed ice was put into a beaker of water and well stirred. This water soon attained a temperature of 0° C., as was shown by a thermometer. I then went into the dark-room and waited there until my eyes could readily perceive the light given out by some fruit-bodies in a small damp - chamber. Miss Irene Mounce, who kindly acted as my assistant, then removed the inverted beaker (*d* in Fig. 173), lifted the test-tube (*a*) from the ice-water in the ice-

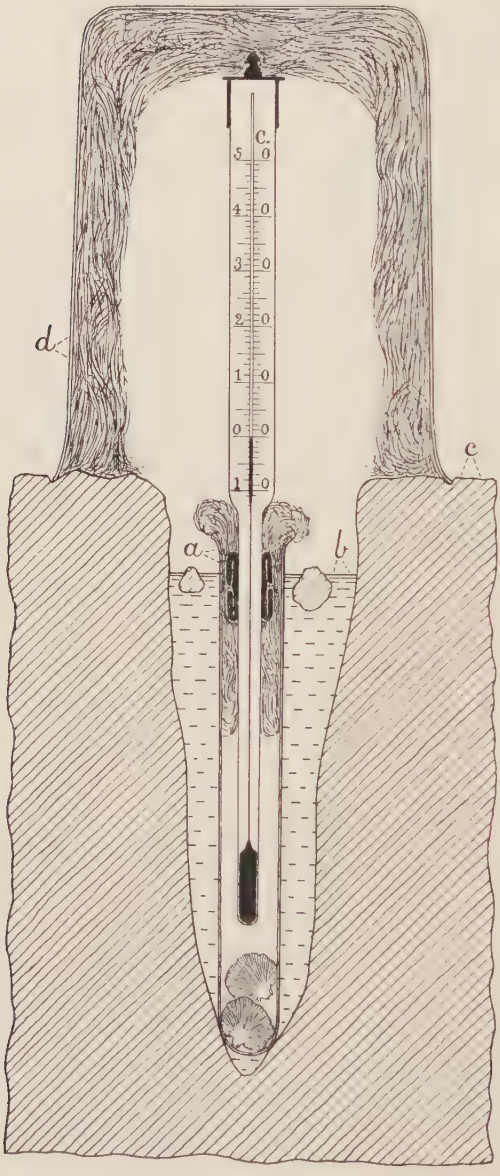


Fig. 173.—Apparatus for subjecting fruit-bodies of *Panus stypticus* p. f. *luminescens* to a temperature of 0° C. The test-tube *a*, fitted with a thermometer held in place with cotton wool and rubber tubing, encloses two fruit-bodies. The ice-water *b* surrounds the test-tube and is contained in a cavity of the ice-block *c* of which only a part is shown. The inverted beaker *d*, lined inside with cotton wool, covers the thermometer and holds the test-tube upright. The fruit-bodies, thus subjected to a temperature of 0° C., continued to give out light. About ⅔ natural size.

block, immersed the test-tube at once in the ice-water of the second beaker, and then immediately brought this second beaker with its contents to me in the dark-room. Thereupon, I brought the test-tube up to the side of the beaker and observed that the fruit-bodies were still luminescent, although faintly. Miss Mounce also soon perceived the light emitted. Shortly after this I took the beaker into the laboratory and observed that the thermometer in the test-tube still registered 0° C. This experiment, which was repeated with variations with other fruit-bodies, clearly proves that the fruit-bodies of *Panus stypt. luminescens* are still faintly luminous at 0° C.

— 3·5° to — 5° C. To observe the effect of temperatures less than 0° C. on luminescence, I exposed some fruit-bodies in the open air at Winnipeg on suitable evenings in December.

Six fruit-bodies, which at the laboratory temperature of about 22° C. were strongly luminous, were placed with gills uppermost in the middle of a large crystallising dish. This dish was then put into a much larger lidless cardboard box lined with black paper, and the whole was then set outside on a flat roof, so that the fruit-bodies were exposed freely to the open sky. The temperature of the air was recorded by means of two thermometers placed in the dish. After the fruit-bodies had been exposed, I made observations for temperature and luminescence as follows. I lifted up the thermometers and read them in the open in a weak beam of light coming through a laboratory window. To observe the fruit-bodies for luminescence in complete darkness, I covered my head and the large cardboard box for a few seconds with a black cloth. To keep my eyes in a sensitive condition I paced about the roof or otherwise avoided artificial light as much as possible.

Observations were begun at 6·37 P.M. on December 7, 1921, and were continued for 2·5 hours. During this period the temperature of the air slowly declined from — 3·5° to — 4·1° C. after one hour, and to — 5° C. after 2·5 hours. The fruit-bodies glowed brightly for the first half hour, feebly at the end of an hour, and not at all at the end of 2·5 hours. The data for time, temperature, and luminescence are embodied in the Table on p. 404.

At the end of the experiment (after 2·5 hours), I covered the dish containing the fruit-bodies with a glass lid which had been

in the open, took the dish into the dark-room the temperature of which was 22° C., and exposed the fungi to the warm air. For 3 minutes after exposure the fruit-bodies emitted no light whatever, but at the end of this time they began to glow feebly and, within 15 minutes after first being exposed, they were glowing with their maximum brilliancy.

A Low Temperature Experiment with Panus stypt. luminescens.

Time in Hours.	Temperature Centigrade.	Degree of Luminescence.
0	− 3·5°	Strong
0·5	− 4°	Bright
1·0	− 4·1°	Feeble
1·5	− 4·5°	Very feeble
2·5	− 5°	Invisible

The experiment just recorded proves that the minimum temperature for luminescence in *Panus stypt. luminescens* is above − 5° C. It also shows that the fruit-bodies only slowly lose their luminescence even at a temperature below − 3·5° C. and that, after exposure to a temperature of − 5° C., they soon recover their full power of emitting light when they are brought into a warm room.

− 1·75° to − 2·7° C. A similar experiment to that just described was begun at 6.30 P.M. on December 10, 1921. Observations were made at intervals for 7 hours, and a final observation after the temperature of the air had sunk to − 10° C. at the end of 16 hours. The data for time, temperature, and luminescence are embodied in the following Table.

A Low Temperature Experiment with Panus stypt. luminescens.

Observation Number.	Time in Hours.	Temperature Centigrade.	Degree of Luminescence.
1	0	0°	Strong
2	1·6	− 1·75°	Bright
3	3·5	− 1·00°	Bright
4	4·5	− 2·25°	Bright
5	5·6	− 1·75°	Bright
6	6·0	− 1·80°	Bright
7	6·75	− 2·70°	Faint
8	16	− 10·00°	Invisible

From the data given in the Table it will be seen that for 5 hours (observations 2–7) the temperature to which the fruit-bodies were exposed varied from — 1° to — 2·7° C. and that during this time the fruit-bodies continued to emit light, although only faintly at — 2·7° C. After midnight the temperature descended from — 2·7° C. and in the morning (observation 8) was — 10° C. At — 10° C. the fruit-bodies were non-luminous. On taking them into the dark-room, as in the previous experiment, their luminescence began to return after about 5 minutes and, in less than half an hour after their exposure to the warm air, they appeared to be glowing with their maximum intensity. Extremely low temperatures, therefore, do not appear to injure the photogen in any way but only to retard its action.

The two foregoing experiments, together with others which I shall not describe, point to the conclusion that in *Panus stypt. luminescens* the minimum temperature for luminescence lies between — 2° and — 5° C.[1]

It may be that the fruit-bodies of *Panus stypt. luminescens* are luminescent until their cell-sap freezes, but this possibility was not investigated.

38° C. Some luminous fruit-bodies were placed in a test-tube containing a thermometer (*cf. a* in Fig. 173, p. 402) and the test-tube was plunged into water at 38° C. contained in a large beaker which was insulated with cotton wool. The beaker contained a thermometer and a looped stirring rod. The apparatus was set in the dark-room. In the dark, by looking through the wall of the beaker, the water in the beaker, and the wall of the test-tube, I was able to observe the fruit-bodies and to watch the changes in their luminosity. The temperature of the water was kept sufficiently constant by adding hot water to the beaker when necessary. As

[1] There are organisms which give out light at still lower temperatures, *e.g.* the Beetle *Lampyris* at — 10° C. (Macaire, 1921), and certain Bacteria: *Pseudomonas javanica* at — 20° C. (Eijkman, 1892); *Bacterium phosphorescens* at — 12° C. (Lehmann, 1889): light bacteria, at — 11·5° C. (Harvey, 1913); and light bacteria at — 7° C. (Tarchanoff, 1902). The power to luminesce, in some organisms at least, is not destroyed by exposure to liquid air; for, on raising the temperature, light again appears (Macfayden, 1902). *Vide* E. N. Harvey, *The Nature of Animal Light*, 1920, pp. 157, 159.

soon as the test-tube was plunged into the water the temperature of the air within immediately began to rise and in the course of a few minutes (certainly after 7 minutes) became equal to that of the water in the beaker outside.

The effect of the increased temperature on the fruit-bodies quickly became obvious. After 3 minutes from the beginning of the experiment the light emitted had become fainter; after 4 minutes it had become very faint indeed; and at the end of 7 minutes it ceased to be emitted, the gills becoming uniformly non-luminous.

Eleven minutes after the experiment was begun, the fruit-bodies were taken out of the test-tube and allowed to cool in the air of the dark-room. The emission of light was resumed but not until about an hour after the cooling had begun, and even then it was very feeble and irregular. Evidently the high temperature of 38° C. had had an injurious effect upon the photogen. From this experiment we may conclude that the maximum temperature for luminescence in *Panus stypt. luminescens* is under 38° C.

42° C. In an experiment similar to the last, but with the water in the beaker at 42° C. instead of 38° C., the light emitted from the fruit-bodies disappeared within three minutes. At the end of this time the thermometer in the test-tube registered 39° C. After 4 minutes from the beginning of the experiment, the fruit-bodies were removed from the hot air in the test-tube and cooled. Light from the gills began to be emitted again about 5 minutes after cooling had begun, but after 15 minutes was feeble relatively to that given out by other fruit-bodies which were used for comparison. After about an hour, however, the intensity of the light seemed to have once more become normal. This experiment shows, therefore, that the exposure of luminous fruit-bodies to a temperature of 39°–40° C. causes the extinction of the light emitted in a very few minutes and that, even when the exposure is very brief, the fruit-bodies are injured so that they do not rapidly recover after being cooled.

30° C. This experiment was conducted in the same manner as the two which have just been described. After 15 minutes the fruit-bodies were still glowing brightly, although perhaps not quite so brightly as they had done at 22° C. After about 30 minutes

the intensity of the light was unchanged. This observation shows that the maximum temperature for luminescence in *Panus stypt. luminescens* is above 30° C.

35° C. Some fruit-bodies, in the same manner as for the three experiments just described, were exposed to a temperature of 35° C. The light from the gills began to grow dim after 6 minutes and was relatively feeble after 12 minutes; but, after an hour from the beginning of the experiment, the fruit-bodies were still very faintly luminous in spots. This experiment therefore indicates that in *Panus stypticus* the maximum temperature for luminescence is about 35° C.

10° C. An experiment with a temperature of 10° C. was carried out by placing cold water in the beaker and by adding colder water when necessary. Fifteen minutes after exposure had begun, the fruit-bodies were still glowing brightly and thereafter continued to glow in an even manner without their photogen showing any trace of injury from the reduced temperature.

Summing up the results of the temperature experiments which have now been recorded, we may conclude that: the maximum temperature for the luminescence of *Panus stypt. luminescens* fruit-bodies is 35° to 37° C., the minimum temperature — 2° to — 4° C., and the optimum about 10° to 25° C.

The following Table embodies the data for temperature as obtained by Kawamura for *Pleurotus japonicus* and by myself for *Panus stypt. luminescens.*

Bioluminescence and Temperature.

—	Pleurotus japonicus.	Panus stypticus luminescens.
Minimum temperature .	3° to 5° C.	— 2° to — 4° C.
Maximum temperature .	40° C.	35° to 37° C.
Optimum temperature .	10° to 15° C.	10° to 25° C.

From the data in the Table it is evident that the maximum and minimum temperatures for the luminescence of *Panus stypt. luminescens* are each several degrees lower than the maximum and minimum temperatures respectively of *Pleurotus japonicus.* The

former fungus appears to be adapted to carry on its functions at a somewhat lower temperature than the latter.

Evidence Proving the Existence of Luminous and Non-luminous Forms of Panus stypticus.—The fruit-bodies of *Panus stypticus* gathered in North America resemble morphologically those gathered in Europe; and, so far as their structure is concerned, there seems to be no justification for regarding them as belonging to two distinct forms. Yet physiologically, as already stated in an earlier Section, the North American fruit-bodies differ from the European, for *the North American fruit-bodies are luminous*, whereas *the European ones are not*. Hence, following precedents in treating of the taxonomy of the Rust Fungi, Bacteria, etc., I have divided the species *Panus stypticus* into two physiological forms: *P. stypt. luminescens* occurring in North America, and *P. stypt. non-luminescens* occurring in England.[1]

The evidence which goes to show that the North American strains of *Panus stypticus* are luminescent, whereas the European strains are non-luminescent, is as follows.

(1) The bioluminescence of *Panus stypticus* was first observed in North America and has never been recorded in Europe.

(2) In North America the bioluminescence of *Panus stypticus* has been observed: (*a*) by Atkinson [2] in fruit-bodies presumably collected near Cornell University, Ithaca (New York State); (*b*) by Bisby [3] in fruit-bodies attached to a stump in a wood near Minneapolis (Minnesota); and (*c*) by myself in fruit-bodies collected at Montreal (Quebec),[4] Ottawa (Ontario), Toronto (Ontario), Ann Arbor (Michigan), and St. Paul (Minnesota).[5]

(3) In England the absence of bioluminescence in fruit-bodies of *Panus stypticus* has been observed: (*a*) by myself, in 1910, in fruit-bodies collected by W. B. Grove near Birmingham; [6] (*b*) by Miss

[1] *Cf.* pp. 365–366.

[2] G. F. Atkinson, *Studies of American Fungi, Mushrooms, edible, poisonous, etc.*, Ithaca, U.S.A., 1901, p. 136.

[3] *Cf.* p. 376.

[4] *Cf.* p. 364.

[5] *Cf.* p. 384. The Ann Arbor and St. Paul fruit-bodies were tested twice, once with collections made in 1921 and a second time with collections made in 1923.

[6] *Cf.* p. 365.

Johnson,[1] in 1920, in pure cultures made from Warwickshire material; (*c*) by myself and other members of the British Mycological Society, in September, 1922, in six collections of fruit-bodies made in various woods at Keswick; (*d*) by Carleton Rea, in 1921, in fruit-bodies collected near Worcester [2] and again, in 1922, in fruit-bodies collected at Keswick; [3] (*e*) by E. M. Corner,[4] in 1922, in fruit-bodies collected at Great Missenden, Buckinghamshire; and (*f*) by myself, in 1922 and 1923, in fruit-bodies collected at Great Missenden and, in 1923, in fruit-bodies collected at West Malvern. All these observations were made with a view to finding out whether the English *Panus stypticus* behaves like the North American in giving out light, but they yielded nothing but negative results. In no case could the faintest trace of light be observed.

At the Keswick Foray of the British Mycological Society, held in the autumn of 1922 and referred to above, I gave a paper on the *Bioluminescence of Panus stypticus* and I was naturally desirous of demonstrating the luminosity of the fungus in a dark séance in the same manner as I had demonstrated it at the University of Manitoba. I therefore collected as many fresh fruit-bodies of *P. stypticus* as possible and took them into a dark-room when my eyes were well rested; but not even after remaining with them in the dark for half an hour could I detect the least sign of light coming from the gills. Dr. Bisby confirmed this negative observation. When giving my paper I was therefore obliged to announce that the English specimens of *P. stypticus*, so far as my experience had gone, behave very differently from North American ones in that they are non-luminous. Subsequently, using five other collections made at Keswick, I found that the fruit-bodies were uniformly dark in a dark-room: no sign of light was emitted from any of their gills.

After hearing my paper at Keswick, Dr. Corner attempted to make photographs with his Great Missenden specimens in the same manner as I had done with fruit-bodies gathered in North America, but without success. He laid about twenty fruit-bodies over the sensitive film of a photographic plate in the dark and left them for

[1] E. M. Johnson, "On the Biology of *Panus stypticus*," *Trans. Brit. Myc. Soc.*, vol. vi, 1920, p. 348.

[2] *In litt.* [3] Communicated in person. [4] *In litt.*

12 hours. During this period the fruit-bodies shed an abundance of spores on the plate but, when the plate was developed, the film was found to be uniformly white, thus showing that it had not been affected by light coming from the fruit-bodies. The experiment was repeated, but again with negative results. Even photographically, therefore, Dr. Corner was unable to obtain any evidence that the English *Panus stypticus* gives out light.[1]

In December, 1922, when visiting Dr. Corner at Great Missenden, I collected a number of fresh fruit-bodies of *Panus stypticus* from a stump in a wood and let them dry out on a table. Subsequently, I took them to Winnipeg. On March 8, 1923, the fruit-bodies were allowed to absorb water through their pilei. They revived, appeared perfectly vigorous, and, within a few hours, began to discharge spores in vast numbers; but, to the eye, in a dark room, they were entirely non-luminous. Some six or seven of the fruit-bodies were placed, with the pilei downwards, upon a clear glass plate and this was then set upon a very sensitive photographic plate with the film turned upwards. This preparation, after being covered to prevent the fruit-bodies from drying up, was left in the dark room for 24 hours. During this period the pilei formed heavy white spore-deposits on the clear sheet of glass (Fig. 174); but they in no way affected the photographic plate beneath, for when this was developed as a negative it was uniformly white.

FIG. 174.—*Panus stypticus* p. f. *non-luminescens*. Spore-deposits made by fruit-bodies resting on a sheet of clear glass which covered a photographic plate for 24 hours in a dark-room. The plate, when developed, showed no signs of having been affected by the fruit-bodies. Photographed in direct sunlight against a black background. Natural size.

The experiments of Dr. Corner and of myself with photographic plates strongly support the view that the fruit-bodies of the English

[1] *In litt.*

physiological form of *Panus stypticus*, which I call *P. stypt. non-luminescens*, give out no light whatever.

In the autumn of 1923, with the assistance of correspondents, I assembled five collections of fruit-bodies of *Panus stypticus*, two from England (West Malvern, Great Missenden) and three from North America (Ottawa, Ann Arbor, St. Paul), and revived the fruit-bodies at one and the same time. The fruit-bodies all resumed their activities and shed an abundance of spores ; but, whereas the three collections from North America became luminous, the two collections from England remained non-luminous. The result of this comparative experiment adds further strength to the view that there are two distinct physiological forms of *Panus stypticus*, one in North America having luminous fruit-bodies and another in England having non-luminous fruit-bodies.

As we have already seen, in *Panus stypt. luminescens* the mycelium shares with the fruit-bodies the property of emitting light. It now remains to add that in *Panus stypt. non-luminescens* the mycelium resembles the fruit-bodies in being entirely non-luminous.

The fact that, in *Panus stypticus*, a mycelium of North American origin is luminous, whereas a mycelium of English origin is non-luminous was demonstrated in one series of experiments as follows. Blocks of Birch wood were sterilised in glass sealing-jars in the manner already described. Some of the blocks were then inoculated with spores from a spore-deposit of North American origin and other blocks were inoculated with spores from a spore-deposit of English origin. The spores germinated and produced mycelia which in the course of a few weeks grew down over the surface of the wood (Fig. 170, p. 391, and Fig. 175). By examination of the jars in a dark room on many separate occasions in the course of a year it was found that every mycelium of North American origin was luminous, while every mycelium of English origin was non-luminous.

The lack of luminosity in a mycelium of English origin was further proved by photographic means. An attempt was made to take a photograph of a fern leaf with English mycelium, using precisely the same method as, with North American mycelium, was

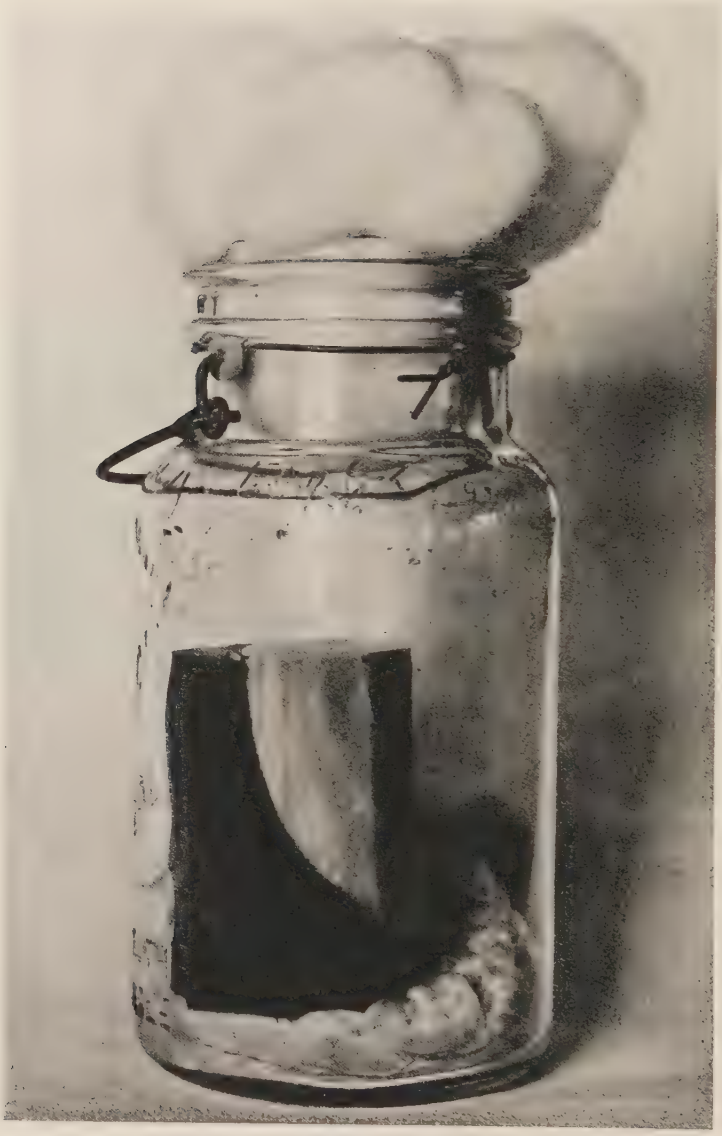


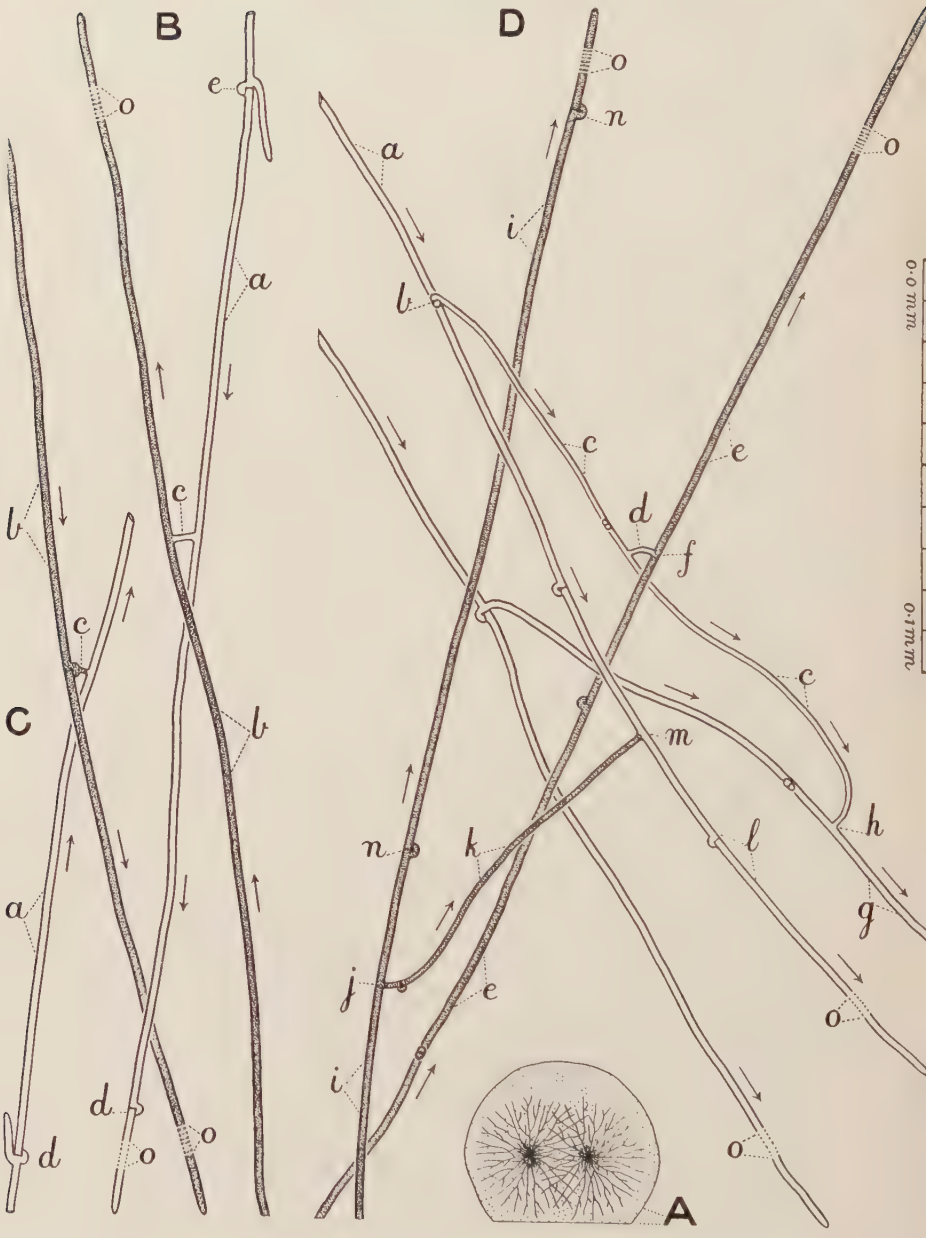
FIG. 175.—Culture of the non-luminous mycelium of *Panus stypticus* physiological form *non-luminescens*. Spores obtained from fruit-bodies gathered at Great Missenden, England, were sown on the top of the sterilised block of Birch wood which rests on wet cotton wool. The mycelium spreading over the block was entirely non-luminous and had no effect on a photographic plate. Photographed by daylight 11·5 weeks after inoculation. About $\frac{2}{3}$ the natural size.

used so successfully in making the photograph of a fern leaf shown in Fig. 171, p. 394. But the attempt was an entire failure ; for the negative, when developed, after an exposure for 24 hours, was uniformly white and showed no trace of a leaf whatsoever.

As may be seen by comparing the photographs shown in Fig. 170 (p. 391) and Fig. 175, the mycelium of North American origin does not differ in external appearance from a mycelium of English origin. Morphologically they are alike, so that one cannot tell them apart by their daylight appearance. Yet, physiologically, they are so different that one glance at them in a dark room is sufficient to enable one to determine their geographical origin.

Hyphal Fusions between the Two Forms.—The mycelia of the two physiological forms of *Panus stypticus* in my cultures all originated from many spores and resembled one another. Both of them were found to consist of very slender hyphae bearing clamp-connections at intervals, the presence of the clamp-connections indicating that the mycelia were in the secondary or diploid phase of their development. It seemed of interest to determine whether or not the mycelia of the two forms would fuse with one another if given the opportunity. Accordingly, two small pieces of mycelium, one piece from *P. stypt. luminescens* and the other from *P. stypt. non-luminescens* were placed near to one another in a hanging drop of nutrient agar. Hyphae from each piece of mycelium grew radially outwards through the culture medium, and soon luminous and non-luminous hyphae began to cross one another (Fig. 176, A). Thereafter, it was observed that hyphal fusions took place between the hyphae of the two physiological forms ; and some of these fusions are shown in Fig. 176, B, C, and D. In this figure, purely for the sake of distinguishing the two forms from one another, the hyphae of the non-luminous form have been shaded, while those of the luminous form have been left unshaded. In B and C, hyphal fusions are shown at *c*, and in D at *f*, *h*, and *m*. The drawings were made with the help of the *camera lucida*.

The observations embodied in Fig. 176 show that the two physiological forms of *Panus stypticus* have mycelia which not only resemble one another in microscopic appearance but which are able to fuse with one another. This affords strong evidence that the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0·1 mm

two forms are very closely related to one another and indeed belong to the same species.

Since, from the recent observations of Mlle Bensaude, Hans Kniep, Miss Mounce, Vandendries, and others, it appears that most Hymenomycetes are heterothallic, it is probable that *Panus stypticus* is also heterothallic. Assuming the truth of this supposition, it would be of considerable interest to obtain mycelia of monosporous origin from *P. stypt. luminescens* and from *P. stypt. non-luminescens*, to cross them, and study their progeny. This would enable one to determine whether or not luminosity and absence of luminosity are a pair of unit characters which are inherited in a Mendelian manner. It would also be of interest to find out which of the two characters, if either, is dominant. It is possible that the secondary (diploid) mycelium resulting from crossing two primary (haploid) mycelia of opposite sex might be (1) always luminous, (2) always non-luminous, or (3) sometimes luminous and sometimes non-luminous. Unfortunately, up to the present, I have not had sufficient leisure to attempt to solve these problems.

Geographical Distribution of the Two Forms.—The geographical limits of the two physiological forms of *Panus stypticus*

Fig. 176.—*Panus stypticus.* Hyphal fusions between luminous and non-luminous forms. A, a hanging drop of nutrient agar, seen through the cover-glass of a ring-cell, in which were planted side by side two mycelial masses, one derived from a North American (Minnesotan) luminous form (*P. stypt. luminescens*), and the other from an English non-luminous form (*P. stypt. non-luminescens*). The illustration shows diagrammatically how the hyphae of the two forms grew through the agar toward one another and crossed. B, C, and D, fusions between a luminous and a non-luminous form as seen in a hanging drop of agar. Illustrations drawn with the *camera lucida*. To distinguish the forms, the hyphae of the non-luminous form have been shaded, while those of the luminous form have been left unshaded. The breaks in the hyphae, at *o o*, indicate that, owing to lack of space, parts of the hyphae have been omitted in the drawing. The arrows everywhere indicate directions of growth of the hyphae. B : a luminous hypha, *a*, and a non-luminous one, *b*, growing in opposite directions, have united by means of the bridging hypha *c* ; *d*, a clamp-connection ; *e*, an older clamp-connection which has given rise to a lateral branch. C : the union of a luminous hypha, *a*, with a non-luminous, *b*, by means of a bridging hypha, *c*, sent out from a clamp-connection ; *d*, a clamp-connection which has just given rise to a lateral branch. D : a luminous hypha *a*, from the clamp-connection *b*, sent out a lateral branch *c* which, by means of a bridging hypha *d*, united with the non-luminous hypha *e* at the point *f*. Continuing its course, the hypha *c*, in response to a chemotropic stimulus, turned toward, and fused with, the luminous hypha *g* at the point *h*. The non-luminous hypha *i*, at the point *j*, gave off a lateral branch *k*, which directed itself toward the luminous hypha *l* and fused with it at the point *m*. Every hypha is now connected directly or indirectly with every other hypha. *n n* are clamp-connections. The scale is 0·1 mm. long. Magnification : A, a little less than 2 ; B, C, and D, 534.

remain to be precisely determined. Up to the present, my observations all point to the conclusion that *P. stypt. luminescens* is widely spread throughout North America and does not occur in England, and that *P. stypt. non-luminescens* is found in England and does not occur in North America. Since luminous fruit-bodies of *Panus stypticus* have never been recorded by any European mycologist, it seems extremely probable that *P. stypt. non-luminescens* is not confined to England but occurs throughout Europe. Possibly its distribution is Eurasian in extent. It seems most likely that one of the two physiological forms arose from the other and that to-day they are separated geographically because the great barrier of the Atlantic Ocean prevents their intermingling.

Luminous and Non-luminous Forms of Xylaria Hypoxylon.—There seems to be no special reason why there should not be as many luminous Ascomycetes as Hymenomycetes ; but, up to the present, the only ascomycetous genus in which bioluminescence has been observed is Xylaria.

In 1874, Ludwig[1] asserted that the mycelium of *Xylaria Hypoxylon* (Fig. 177) contained in rotting wood obtained from stumps is luminous ; and, in 1881, Crié,[2] apparently without knowing of Ludwig's paper, made a similar claim for *X. polymorpha* (Fig. 178). However, in 1904, Molisch,[3] after studying *X. Hypoxylon* and *X. Cookei* in pure cultures, was unable to confirm Ludwig's and Crié's observations : he grew *X. Hypoxylon* in pure cultures for four years ; but, during all this time, neither the mycelium nor the fruit-bodies ever gave out the faintest trace of light. He therefore came to the conclusion that Ludwig and Crié had probably been mistaken in the identification of the mycelium which they had had under observation.

In 1907, Guéguen,[4] apparently without knowing of the observations of Molisch, published an account of the biology and anatomy

[1] F. Ludwig, *Ueber die Phosphorescenz der Pilze und des Holzes*, Inaugural Dissertation, Berlin, 1874.

[2] L. Crié, "Sur quelques nouveaux cas de phosphorescence dans les végétaux," *Compt. Rend.*, 1881, p. 853.

[3] Hans Molisch, *Leuchtende Pflanzen*, Jena, 1904, pp. 40–44.

[4] M. F. Guéguen, "Recherches biologiques et anatomiques sur le *Xylaria Hypoxylon*," *Bull. Soc. Mycol. France*, T. XXIII, 1907, pp. 197–198.

of *Xylaria Hypoxylon*, in which he asserted that, after having made pure cultures of the fungus on various media, he was able to confirm Ludwig's statement that the fungus is luminous. "I too" says Guéguen "have had occasion to observe this phosphorescence in Xylarias kept under a bell-jar, as well as in the mycelium obtained in cultures shortly to be described. The phosphorescence, as Ludwig remarked, is exclusively confined to the mycelium. In both instances it seemed very feeble and in no way comparable in intensity with that one observes so frequently during the warm season on fish and other marine animals exposed to the air. The glow of the Xylaria is white tinged with blue, and one can only perceive it clearly in complete darkness. It is only to be observed at certain periods in the life of the fungus, *e.g.* when the mycelium is in full development. Old cultures no longer exhibit it."

FIG. 177.—Fruit-bodies of *Xylaria Hypoxylon*, the Stag's-horn Fungus, growing from the bark of a dead tree. The white and bifurcated ends of the branches are covered with conidiophores which produce vast numbers of conidia. The latter disperse as a visible cloud when the branches are suddenly jarred. The black lower parts of the branches are persistent and develop beneath their surface numerous perithecia containing asci and ascospores. The mycelium of the fungus, grown in pure cultures, was found by Guéguen to be luminous and by Molisch to be non-luminous. Photographed in Yorkshire, England, by A. E. Peck. Natural size.

It thus appears that, as a result of studies with pure cultures,

Molisch came to the conclusion that *Xylaria Hypoxylon* is non-luminous and Guéguen that the fungus distinctly gives out light. How shall we harmonise these diametrically opposite results? It is possible that Molisch and Guéguen were both working with essentially the same form of the fungus and that the difference in their results was due to a difference in the composition of their culture media.[1] However, it may be that the two workers employed two different physiological forms, that of Guéguen being a luminous one and that of Molisch non-luminous. According to this explanation, the French and German forms of *Xylaria Hypoxylon* correspond respectively to the North American and English forms of *Panus stypticus*, in that the former has an internal metabolism which results in bioluminescence, whereas the latter has not. To determine with absolute certainty whether some forms of *X. Hypoxylon* are luminous while others are not, it will only be necessary to

FIG. 178.—A fruit-body of *Xylaria polymorpha*. The swollen portions, just beneath the surface, contain numerous perithecia which emit ascospores through fine ostioles opening on the exterior. In 1882 Crié asserted that the mycelium of this fungus is luminescent. Photographed by A. E. Peck at Scarborough, England. Natural size.

[1] That the medium upon which the mycelium grows may affect the intensity of the light given out by a luminous fungus is shown by some facts recently communicated to me by Dr. A. W. McCallum of Ottawa. Dr. McCallum found that the mycelium of *Armillaria mellea* was "strongly phosphorescent when growing on spruce wood and bread soaked in prune juice, and apparently not so when grown on such media as potato and corn-meal agars." But he adds: "Recently, however, I have observed that this fungus is faintly luminous on potato-dextrose agar." It

grow as many forms as possible under the same conditions in the laboratory and observe the results.

The Bioluminescence of Decaying Leaves.—The bioluminescence of decaying leaves has been observed by Tulasne in France, by Molisch in Java and Germany, by myself in England, and by Gilbert, Dosdall, and others in North America. As this phenomenon is geographically so wide-spread, intrinsically so interesting, and yet so little known even to mycologists, I shall here review our knowledge concerning it.

Tulasne,[1] in 1848, in France, discovered that decaying Oak leaves sometimes emit light. At that time he was investigating the emission of light from *Rhizomorpha subterranea*, which we now know is not an independent species but merely a specialised part of the mycelium of *Armillaria mellea*. He gathered some rhizomorphs in a wood and, to protect them from drying during transit to his laboratory, wrapped them up with damp leaves and small twigs of an Oak. On examining his material in the dark, he at once perceived that light was being emitted not only by the rhizomorphs but also by the leaves. The leaves were those of the previous year which had fallen in autumn, and they were damp, elastic, and still somewhat firm. They did not give out light over their whole surface but only in spots, more especially where the brown leaf-colour had already become pale or whitish.

For fifty years after the phenomenon of bioluminescence in dead leaves had been discovered by Tulasne, it was neglected by botanists. Then Molisch [2] resumed its study, and in 1904 he published the results of his investigations in his *Leuchtende Pflanzen*. On his visit to the Botanical Garden at Buitenzorg he found that well-rotted leaves of Bambusa, Nephelium, Aglaia, etc., gave out a white

is also known that the composition of the medium affects the luminosity of certain Bacteria. Thus a luminous species which parasitises and kills the Sand-hopper *Talitrus* (an amphipod Crustacean) is non-luminescent when grown on ordinary agar, gelatine, or potato media, but resumes its luminescence when inoculated into a healthy *Talitrus* (*vide* Paul Buchner, *Tier und Pflanze in intrazellularer Symbiose*, Berlin, 1921, p. 342).

[1] L. R. Tulasne, "Sur la phosphorescence spontanée de *l'Agaricus olearius* DC., du *Rhizomorpha subterranea* Pers. et de feuilles mortes du chêne," *Annal. d. scienc. nat.*, sér. 3, Tab. IX, 1848, p. 353.

[2] Hans Molisch, *Leuchtende Pflanzen*, Jena, 1904, pp. 47–51.

light and, if kept damp, continued to give out light for several weeks. Afterwards, in Germany, he found that bioluminescence was frequently exhibited by rotting leaves of the Beech (*Fagus silvatica*), Oak (*Quercus*), Hornbeam (*Carpinus Betulus*), and Sycamore (*Acer Pseudoplatanus*). A microscopical investigation proved that those parts of the leaves which radiated light contained a weft of brown or colourless fungus hyphae. The emission of light by dead leaves was therefore attributed by Molisch to fungi. The identification of the species concerned was attempted by Molisch, but unsuccessfully; and this attractive problem, which involves the making of pure cultures and the development of fruit-bodies, still remains unsolved.[1]

In giving directions as to the best means of finding luminescent leaves, Molisch [2] says: "One should seek especially in those places where the leaves of the previous year and of earlier years lie in a thick layer about 10–30 cm. (4–12 inches) deep. The uppermost leaves are mostly dry, brown, and of a firm consistence. Under these there is often a zone of leaves which are like the leaves of a book in that they lie flattened one upon the other, although irregularly. These leaves are already in an advanced state of decay and are distinguished by their more yellow or whitish-yellow colour which can be seen throughout the whole leaf or only in spots. . . . It is chiefly the bright spots standing out on the deeper brown of the leaves which give out light, and their presence indicates the degree of decay which is most favourable for luminescence. If one collects a few handfuls of such leaves, one can be sure that at night one will be able to observe them glowing with a light which is more or less clear and not infrequently splendid."

To the above Molisch [3] adds the following remarkable statement: "Now that I have known the phenomenon (of the luminescence of decaying leaves) for five years and have assured myself of its general prevalence I can say, without fear of exaggeration, that in an Oak wood or Beech wood a by no means inconsiderable

[1] I have seen fungus hyphae in luminous leaves but, doubtless, they are accompanied by numerous bacteria. Whether the luminescence is caused by fungi or by bacteria seems to me to be as yet an open question.

[2] Hans Molisch, *Leuchtende Pflanzen*, Jena, 1904, pp. 50–51.

[3] *Ibid.*, p. 51.

fraction of the fallen leaves are in a condition in which they give out light, and that the forest floor everywhere is receiving radiation from the light of decaying leaves." Molisch expresses astonishment that so wide-spread a phenomenon as the bioluminescence of decaying leaves should be practically unknown to foresters, woodmen, botanists, etc., and he presumes that this is simply due to the fact that most people avoid woods at night.

It seemed of interest to find out whether or not luminous leaves occur in England in the same manner as in Germany. In the years 1922 and 1923, therefore, I made the following series of observations.

About June 1, 1922, at King's Heath, a suburb of Birmingham, I went out into my father's garden at night and, covering my head and a heap of old Sycamore leaves with a coat, examined the leaves for luminescence. One of the petioles gave out a bright light, but no other signs of luminosity could be observed. In the same garden I also examined a heap of Beech leaves which had been raked together the previous autumn. Within it I found a few luminous rhizomorphs of *Armillaria mellea* and some tiny insects which gave out light when disturbed and which I took to be species of Collembola, but no luminous leaves. However, the conditions under which the heap of leaves had been made were artificial. I therefore determined to turn my attention to the leaf-mould in a wood.

On June 10, I visited a wood on the border of Sutton Park, Warwickshire. In a hollow underneath some large Beech trees, which were about 150 years old, there was a thick layer of leaf-mould that had evidently been accumulating for some years. I removed the uppermost, relatively dry, curled-up leaves and filled a small attaché case with the moister, more flattened leaves thus exposed. These had fallen in the previous year and were brown, elastic, and still almost intact ; but they showed signs of decay in that to a certain extent they were discoloured with somewhat pale or yellowish blotches. To the Beech leaves I added a few Oak leaves obtained from the mould beneath an Oak tree (*cf.* Fig. 179). The same evening I emptied out the leaves upon a large tray in a dark-room and examined them for luminescence. As soon as my eyes had become adapted to the darkness, I perceived that a number

FIG. 179.—Three luminous Oak and two luminous Beech leaves obtained from a forest floor at Four Oaks, Warwickshire, England. The right top Oak leaf was luminous all over, while the luminosity in the other leaves was confined to their lighter and more decayed parts. The leaves fell in the autumn of 1922 and were collected July 7, 1923. Natural size.

of the leaves were luminous over nearly their whole surface or in patches. I soon picked out thirty Beech leaves and one Oak leaf all of which gave out a soft whitish light. The light emitted was not very bright, but it enabled me to distinguish the luminous from the non-luminous leaves with ease and certainty. Thus, at my very first attempt, I succeeded in obtaining luminous leaves from forest mould.

On June 17, I filled my attaché case with decaying leaves taken from the mould lying beneath certain trees in Kew Gardens. On examining this booty at night in the dark, I found that many of the Oak and Beech leaves were beautifully luminous.

On August 4, I gathered a few handfuls of decaying Oak leaves from beneath some trees in a wood at Tanworth-in-Arden, Warwickshire. A number of the leaves, when examined in the evening in the dark, were found to give out a soft white light. The light came from the whiter more highly decayed parts of the laminae and not from the less decayed browner parts.

In the third week of September, whilst attending the Keswick meeting of the British Mycological Society, I made three small collections of Oak leaves from as many different Cumberland woods. Some of the leaves in each collection, especially those exhibiting discoloured whitish or yellowish decay-spots, gave out light. At a dark séance held at the close of one of the Society's evening meetings, I demonstrated luminosity of the leaves to the members in attendance.

In July, 1923, in a wood at Four Oaks, Warwickshire, I collected a large number of luminous leaves from the leaf-mould. Most of them were Beech and Oak leaves but of the remainder some undoubtedly belonged to the Spanish Chestnut (*Castanea vesca*) and others to a cultivated Rhododendron. Some of these leaves are shown in a photograph reproduced in Fig. 179.

As a result of the above recorded observations in Warwickshire, Surrey, and Cumberland, there can be but little doubt that the phenomenon of bioluminescence in decaying leaves is of common occurrence throughout the woods of England.

As we have seen, luminous leaves have been found in France, Germany, England, and Java. It seemed to me of interest to find

out whether or not luminous leaves occur in North America. Whilst resident in England during the summer and autumn of 1922, I therefore wrote to several botanists in Canada and the United States requesting them to look for such leaves and report to me the results of their investigations. The reports which my correspondents were good enough to send prove that the phenomenon of bioluminescence in decaying leaves undoubtedly occurs in the United States.

Dr. E. M. Gilbert of the University of Wisconsin investigated rotting leaves in the neighbourhood of Madison (Wisconsin) and reported as follows :

" During the early part of September we had several days when rainfall was sufficient to moisten thoroughly the soil in our denser woods. On September 14 and again on September 15, I made collections of leaves as suggested and placed them on a bench in one of our dark-rooms. Returning to the room after a few minutes, I saw that there was a distinct emission of light from certain of the collections. So much light was given off that five of the nine lots could be easily located. I picked out from each lot the leaves which were luminescent and took them into daylight. I then found that almost all of them were oak leaves."

Miss Louise Dosdall of the University of Minnesota investigated rotting leaves at the University Farm, St. Paul (Minnesota), and reported as follows :

" On July 7, I went into an oak wood just off the Agricultural College campus and filled a vasculum with moist decaying oak leaves. The upper leaves were dry and I discarded them, taking only the moist well decayed ones below. These were firm and still recognisable as oak leaves, although many of them broke when handled. White spots and heavy mats of fungus hyphae were common on the leaves.

" After dark, I examined my booty in the laboratory and found many of the leaves beautifully luminescent. In some leaves only the petioles or mid-ribs gave out light, but in others there were large luminescent spots. By marking the spots in the dark, I found that it was always the greyish-white spots on the leaves which were luminescent and that the heavy mats of white mycelium

emitted no light whatever. The light was pale white. I picked out at least twenty leaves with luminescent spots, and the light from some of the larger ones was sufficiently strong to enable me to see the outline of my hand.

"I was so pleased with the results that on July 11 I gathered several vasculums full of oak leaves from the same woods and gave a demonstration of the phenomenon at the close of our literature seminar. On this occasion we found a great many luminescent leaves and, as before, the light seemed to come from the greyish-white spots. We also found growing among the leaves many rhizomorphs, and these gave off a brighter light than the leaf-spots.

"From these two trials, I can assure you that luminescent oak leaves are very common here. They are indeed so common that everyone has been wondering why he had not discovered them for himself long ago."

The above reports of Dr. Gilbert and of Miss Dosdall prove in the most convincing manner that the bioluminescence of decaying leaves is a common phenomenon in certain woods in the United States.

The reports from my Canadian correspondents were all negative. Those who failed to find luminous leaves in Canada were as follows: (1) Miss Irene Mounce and Mr. C. W. Lowe at Vancouver, British Columbia; (2) Mr. C. W. Lowe at Winnipeg, Manitoba; (3) Dr. Margaret Rioch at Kenora, western Ontario; and (4) Mr. F. L. Drayton at Ottawa, eastern Ontario.

Mr. Drayton in eastern Ontario examined the following six kinds of decaying leaves: (1) Yellow Birch, *Betula lutea*, (2) Iron-wood, *Ostrya Virginiana*, (3) Sugar Maple, *Acer Saccharum*, (4) Blue Beech, *Carpinus caroliniana*, (5) White Spruce, *Picea canadensis*, and (6) White Oak, *Quercus alba*; but in none of them could he detect the least trace of luminescence.

At Winnipeg, in the spring of 1923, I failed to find any luminous leaves among Oak-leaf mould, but the absence of bioluminescence may have been due (1) to the leaves of the previous autumn not being sufficiently rotted and (2) to the leaves of the penultimate autumn being rotted too much. The leaf-mould at Winnipeg is very thin and therefore contrasts strongly with the thick leaf-mould

one is used to observing under Oaks and Beeches in England. Possibly, where the leaf-mould is very thin, bioluminescence is absent; but, before concluding that luminous leaves do not occur in the woods around Winnipeg, one ought to investigate the rotting leaves of the Oak at successive intervals of time throughout the summer.

Notwithstanding the negative observations just recorded, it seemed highly probable that luminous leaves occur in Canadian woods in the same manner as in woods in the United States. I therefore made some further enquiries, and these have yielded positive results. It appears that, in eastern Canada, luminous leaves have been observed from time to time, quite casually, by persons camping out during their summer holidays. Among these campers are: A. D. Temple, a student at the University of Manitoba, and Dean E. M. Freeman of the University of Minnesota.

Mr. Temple, who had never previously heard of luminous leaves, discovered them for himself. He makes the following statement: "While walking in the woods at De Grassi Point, Lake Simcoe, Ontario, one night toward the end of August, 1922, I noticed some leaves which appeared to be glowing. I picked one up, and could distinctly see the outline of the leaf, although it was a very dark night."

Dean Freeman[1] thus recounts his experience: "While on a canoe trip through the Ontario Quetico Provincial Forest, we camped on the north shore of one of the arms of Sturgeon Lake in a grove of poplars and birches. In the evening one of the party casually disturbed some of the leaves in a fairly thick bed of dead leaves on the floor and noticed a very pronounced luminescence. He called the matter to the attention of the party and, upon further search, it was found that the luminescence was very wide-spread throughout the locality where our camp was pitched. The glow of the leaves could be observed at some distance and was a beautiful phenomenon."

On the basis of personal observation and enquiry as recorded above, I am now able to affirm that luminous leaves, in addition to occurring in France, Germany, and Java as reported by

[1] *In litt.*

Tulasne and Molisch, also occur in England, Canada, and the United States. As time goes on, doubtless, many other countries will be added to this list, and it is even possible that luminous leaves may be found in broad-leaf forests throughout the whole world.

Persistence of Luminescence in Leaves.—One midnight in July, 1923, whilst in England, I collected a large number of luminous leaves (Oak, Beech, Spanish Chestnut, Rhododendron) from a wood

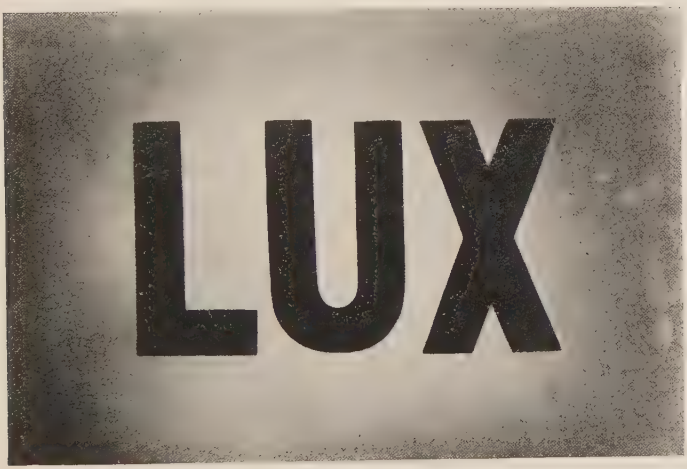


Fig. 180.—A shadow-photograph of black-paper letters made with the light emitted by luminous Beech, Oak, and Spanish Chestnut leaves. Leaves collected at Four Oaks, Warwickshire, England. Exposure of negative, 50 hours. Original size.

at Four Oaks, Warwickshire (Fig. 179, p. 422). The wood was so dense, the night so dark, and the leaves glowed so brightly, that I was able to select the leaves from the leaf-mould by the amount of light which they emitted. The leaves were allowed to dry and were then transported to Winnipeg. After remaining dry and non-luminescent for ten months, some of the leaves were set in a large covered crystallising dish and sprayed with water. Within a few hours a number of the leaves became luminescent once more. The leaves were kept moist and it was observed that, at room temperature, they continued to give out their light for 7–8 weeks. The bioluminescence of decaying leaves, under favourable laboratory conditions, is therefore a very persistent phenomenon. In nature,

doubtless, its continuance is often interrupted by periods of drought.

Photographs made with the Light of Luminous Leaves.—Light from the re-moistened luminous leaves just described was used for making the shadow-photograph shown in Fig. 180. Some black-paper letters making up the word LUX were stuck on to a clear glass plate and this was placed in a printing frame over a photographic plate, so that the letters pressed against the film. A small crystallising dish containing about half a dozen luminous leaves

Fig. 181.—A shadow-photograph of an Indian ink drawing on a sheet of glass, made with the light emitted by luminous Beech, Oak, and Spanish Chestnut leaves collected at Four Oaks, Warwickshire, England. Exposure of negative, 50 hours. Original size.

was placed in the middle of a much larger crystallising dish provided with a cover. The printing frame was then set as a cover over the inner small crystallising dish in such a way that the sensitive film was directed toward the luminous leaves. The distance between the leaves and the film was about one inch. The plate was exposed to the light of the leaves for 50 hours and then developed. The negative yielded a print which is reproduced in Fig. 180. When the exposure of the negative was reduced to 24 hours, the resulting print was good, but not so good as that shown in Fig. 180.

Another shadow-photograph made with the light of the

luminous leaves in the manner just described is reproduced in Fig. 181. The design was drawn with Indian ink on a sheet of clear glass and, when this was placed in the printing-frame, the dried ink was in contact with the sensitive film of the photographic plate. As before, the negative was exposed to the light emitted by the leaves for a period of 50 hours.

Remarks on the Bioluminescence of Armillaria mellea.—In the forests of Europe and North America *Armillaria mellea* (Fig. 38, p. 87; also Vol. II, Fig. 73, p. 203) is an exceedingly common fungus; and there can be no doubt that, in these regions, its mycelium is the chief cause of the production of luminous wood. For some general remarks on *A. mellea*, including observations on some luminous wood found on the shore of Lake Winnipeg, the reader is referred to Chapter IV.[1]

It is a striking fact that in *Armillaria mellea*, while the mycelium invading the cells of the organic substratum and the rhizomorphs are luminescent, the fruit-body is perfectly dark. This restriction of the property of luminescence to the vegetative parts of *A. mellea*, so often noticed by field mycologists, also holds true under laboratory conditions. Thus Molisch,[2] who beginning with spores raised the sporophores of *A. mellea* in pure cultures, says: "It is remarkable that the fruit-body (Hut) of the Honey Fungus (Hallimasch), which nevertheless springs from the dark-coloured mycelial cords of the rhizomorpha, gives out no light at all."

It is true that, as a rule, the fruit-body of *A. mellea* emits no light whatever from either its outer or inner tissues. Yet, on one occasion, in certain fruit-bodies collected in Canada, I observed that a perceptible amount of light was given out by the interior hyphae of the lower part of the stipe. The conditions under which this unexpected observation was made will now be recorded.

On October 14, 1921, at Kenora, central Canada, I gathered some fresh fruit-bodies from a stump, took them to a dark room at mid-day, and carefully examined them for luminescence; but they did not give out the faintest appreciable amount of light. I thereupon split a few of them down the middle, wrapped them up in moist newspaper, and left them until 7 P.M. At that time, a walk

[1] Pp. 86–89. [2] Hans Molisch, *Leuchtende Pflanzen*, Jena, 1904, p. 28.

through the woods in the dark having rendered my eyes very sensitive to weak light, I re-examined the fruit-bodies ; and, to my surprise, I found that the interior part of each stipe-half was luminous for a distance of 0·25–0·5 inch upwards from the very base of the stipe. The unsplit stipes were non-luminous.

During the night following the observation just described there was a sharp frost, and next day I could find only one more group of Armillaria fruit-bodies that was still in good condition. I gathered some of these fruit-bodies, cut them down the centre, took them to Winnipeg, and put them in a moist crystallising dish. At night, just as before, I perceived that the internal basal parts of the stipes were luminous. The luminosity was faint and extended upwards from the base of each stipe for a distance about equal to the breadth of my thumb.

Kawamura [1] observed that the luminescence of *Pleurotus japonicus* is confined to the gills. On the other hand, Tulasne [2] states that in *Pleurotus olearius* the luminescence "is not exclusively confined to the hymenial surface," and he adds: "Numerous observations prove that the whole of the substance participates very frequently, if not always, in the faculty of shining in the dark." Tulasne split a stipe of *P. olearius*, bruised it, and divided it into small fragments, but yet found that "the whole of the mass even in its deepest parts enjoyed in a degree similar to its superficies the property of light." It is evident that the fruit-bodies of the central-Canadian strain of *Armillaria mellea*, upon which my observations were made, differ from the fruit-bodies of *Pleurotus japonicus* and *P. olearius* in that their luminescence was confined to the inner tissues of the base of the stipe.

In the summer and autumn of 1922, whilst resident in England, I examined in the dark the interior of the stipes of a number of *Armillaria mellea* fruit-bodies gathered at King's Heath and Barnt Green in Worcestershire and at Keswick in Cumberland, but in no instance could I detect the faintest trace of light. In 1923, similar

[1] S. Kawamura, "Studies on the Luminous Fungus, *Pleurotus japonicus*," *Journ. of the College of Sci., Imper. Univers. of Tokyo*, vol. xxxv, 1915, pp. 6–9.

[2] L. R. Tulasne, *loc. cit.* (*vide* p. 419); also Cooke and Berkeley, *Fungi, etc.*, London, 1882, p. 106. These authors have translated several pages of Tulasne's description of *Pleurotus olearius*.

observations made by myself at Kenora and by Dr. A. W. McCallum [1] at Ottawa also yielded negative results.

Summing up the evidence that has accumulated so far, it appears that the emission of light by the interior hyphae of the base of the stipe in *Armillaria mellea* is a phenomenon which takes place only under very exceptional conditions.

It is well known that the relatively loose hyphae in the central part of the stipe in agarics generally are but little specialised and can readily be employed for making pure cultures. Doubtless, if one were to inoculate sterilised bread or some other suitable culture medium with hyphae taken from the interior of a stipe of any Armillaria fruit-body, whether obtained in North America or Europe, one would soon obtain a mycelium exhibiting luminescence.

Directions for obtaining luminous wood are as follows. In the late summer or autumn, go into the forest and find a clump of *Armillaria mellea* fruit-bodies on a stump or log. Then with a strong knife, or more easily with an axe, remove some of the subjacent, white, mycelium-containing, rotten wood. Keep the pieces of wood damp and take them home. Examine them at night. One will then readily observe them glowing with an unmistakable soft whitish light. If there are no fruit-bodies in the forest, one should search beneath the bark of rotten stumps for the black shoe-string-like rhizomorphs ; for these belong to *Armillaria mellea* and, like the fruit-bodies, give a clue to the presence of luminous wood. Moreover, the rhizomorphs, if growing, may themselves be luminous.

[1] *In litt.*

CHAPTER XIII

AGARICS WHICH ARE PARASITIC ON OTHER AGARICS

Non-basidiomycetous Fungi Parasitic on Other Fungi—Basidiomycetous Fungi Parasitic on Other Fungi—*Boletus parasiticus*—*Leptonia parasitica*—*Claudopus subdepluens*—*Volvaria Loveiana*—The Genus Nyctalis—*Nyctalis asterophora*—*Nyctalis parasitica*—*Stropharia epimyces*—*Collybia dryophila* and its Pseudo-parasite *Tremella mycetophila*—A Morchelloid Modification of *Collybia velutipes*

Non-basidiomycetous Fungi Parasitic on Other Fungi.—All groups of Fungi, and also Lichens, have their fungus enemies. These fungi which are parasitic on other fungi are of considerable interest, both in themselves and for the elucidation of problems in parasitology; but since, directly, they are of little or no economic importance, phytopathologists tend to neglect their study and scarcely mention them in their text-books.

The basidiomycetous fungi which are parasitic on other fungi are but few. One of them, *Stropharia epimyces*, has received our attention in Chapter VII; and the others will be treated of in the present Chapter.

The non-basidiomycetous fungi which are parasitic on other fungi are far more numerous than the basidiomycetous, and the accompanying selected list of them will perhaps suffice to suggest their number and variety and the wide field they offer for further investigation.[1]

Basidiomycetous Fungi Parasitic on Other Fungi.—Among the Basidiomycetes there are but few species that attack other living fungi, and they are all Hymenomycetes which live on the fruit-bodies

[1] W. Zopf, in 1890 (*Die Pilze*, Breslau, pp. 269–281), gave the names of all the species of fungi which were then known to be parasitic on other fungi, and to his pages I am largely indebted for the data embodied in the Table.

of other Hymenomycetes or of certain Gastromycetes. While, therefore, there are a number of Ascomycetes (Hypomyces, etc.) which parasitise Basidiomycetes, there are no known Basidiomycetes which parasitise Ascomycetes.

Non-basidiomycetous Fungi Parasitic on other Fungi.

Groups to which the Host-fungi belong.	Host-fungi.	Parasitic Fungi.	Authority.
Mucorineae	Mucor mucedo	Piptocephalis Freseniana	Brefeld
		Chaetocladium Jonesii	
	Pilobolus crystallinus	Mortierella polycephala	van Tieghem
Saprolegnieae	Achlya polyandra	Rosella simulans	A. Fischer
	Achlya racemosa	Olpidiopsis incrassata	Cornu
		Rhizidiomyces apophysatus	Cornu
		Rhizidium leptorrhizum	Zopf
	Saprolegnia Thuretii	Olpidiopsis Saprolegniae	Cornu
		Woronina polycystis	Cornu
		Rozella septigena	Cornu
	Monoblepharis polymorpha	Rozella Monoblepharidis polymorphae	Cornu
Fungi Imperfecti	In conidia of Cephalothecium roseum	A species of Monadina (?)	Zopf
	Botrytis Bassii	Melanospora parasitica	Kihlmann
	Isaria farinosa		
	I. strigosa		
Erysipheae	Erysiphe Tuckeri	Cicinnobolus Cesatii	de Bary
Pyrenomycetes	Species of Xylaria	Nectria episphaeria	*vide* Zopf
	Diatrype, Valsa		
	Cucurbitaria, etc.		
Discomycetes	Humaria carneosanguinea	Melanospora Didymariae	Zopf
	Peziza testacea	Bactridium Helvellae	Berkeley and Broome
	P. cochleata	B. acutum	Berkeley and White
	P. macropus	Mycogone cervina	Ditmar
	P. flavo-brunnea	M. Pezizae	Saccardo
	Spathularia flavida	Hypocrea alutacea	Broome
	Helvella infula	Sphaeronaemella Helvellae	Karsten
Tuberaceae	Elaphomyces granulatus	Cordyceps ophioglossoides and C. capitata	Tulasne
	E. variegatus		
	E. muricatus		

[*Continued on next page.*

Non-basidiomycetous Fungi Parasitic on other Fungi—continued.

Groups to which the Host-fungi belong.	Host-fungi.	Parasitic Fungi.	Authority.
Lichenes	Parmelia stellaris	Sclerococcum sphaerale	Various, *vide* Zopf
		Illosporium corallinum	
	Peltigera canina	Phragmonaevia Peltigerae	
		Nectria lichenicola	
		Acanthostigma Peltigerae	
		Phyllosticta Peltigerae	
		Pleospora Peltigerae	
		Hendersonia lichenicola	
	Usnea barbata	Abrothallus Smithii	
		Epicoccum Usneae	
Uredineae	In aecidia of : P. Poarum	Tuberculina persicina	Gobi
	Aecidium Clematidis		
	Gymnosporangium juniperinum		
	In sori of Puccinia Vincae	Tuberculina Sbrozzii	Grove
	In uredo-sori of Puccinia coronata, etc.	Darluca Filum	Fuckel
	In teleutospore-sori of Uromyces Cytisi, etc.		
	In uredospores only	Chytridium Uredinis	Lagerheim
Hymenomycetes	Stereum hirsutum	Hypomyces aureonitens	Plowright
	Clavaria cristata	Pleospora Clavariarum	Tulasne
	C. rugosa		
	C. ligula	Hypocrea alutacea	
	Hydnum species	Hypomyces rosellus	Saccardo
		Hypocrea parasitans	
		Hypoxylon hydnicolum	
	Psalliota campestris	Mycogone perniciosa	Various
	Lactarius piperatus	Hypomyces lactifluorum	
	Paxillus involutus	Hypomyces chrysospermus	Tulasne
	Boletus scaber		
	B. edulis, etc.		
	Polyporus adustus	Melanospora lagenaria	*vide* Zopf
	P. betulinus	Eleutheromyces subulatus	Fuckel
Gastromycetes	Scleroderma verrucosum	Hypomyces chrysospermus	Tulasne
	Melanogaster variegatus		
	Octaviana asterosperma		

The parasitic Hymenomycetes vegetate in the fleshy fruit-bodies of the fungi which they attack and, when they fructify, there is presented to the eye the curious and unusual spectacle of the fruit-body of one agaric growing upon that of another agaric. The following is a list of the hymenomycetous agarics which are parasitic on other agarics :

Hymenomycetous Agarics Parasitic on Other Agarics.

Boletus parasiticus	on	Scleroderma vulgare S. verrucosum
Leptonia parasitica	on	Cantharellus cibarius
Claudopus subdepluens	on	Polyporus perennis
Volvaria Loveiana	on	Clitocybe nebularis C. clavipes
Nyctalis asterophora	on	Russula adusta R. nigricans Lactarius piperatus L. vellereus Collybia fusipes
Nyctalis parasitica	on	Russula adusta R. nigricans R. foetens R. chloroides R. delica Lactarius vellereus L. blennius
Stropharia epimyces	on	Coprinus comatus C. atramentarius

Boletus parasiticus.—*Boletus parasiticus* (Fig. 182) attacks the fleshy fruit-bodies of *Scleroderma vulgare* (= *S. aurantium*) and *S. verrucosum* in England (where I have met with it), France, and other parts of Europe; and it has also been found upon *S. vulgare* in North America.[1] The pileus is 2–8 cm. in diameter, dingy yellow, tawny, or more or less brown, dry, and minutely tomentose ; while the pileus-flesh, hymenial tubes, and stipe are all more or less

[1] C. McIlvaine, *One Thousand American Fungi*, Indianapolis, 1900, p. 427.

yellow. The fruit-bodies arise not from the top but from the base of the host fruit-body, either singly or several together. Coloured illustrations of the fungus are to be found in Berkeley's *Outlines of Fungology*[1] and in Boudier's splendid *Icones Fungorum*.[2]

Boletus parasiticus is the only member of the Polyporeae known to be parasitic on another fungus. The fruit-bodies of the Sclerodermas which it parasitises are well fitted to afford it a nutrient

FIG. 182.—*Boletus parasiticus* as a parasite on *Scleroderma vulgare*. From M. J. Berkeley's *Outlines of British Fungology*. Natural size.

substratum, owing to their common occurrence in woods and heathy places, their massiveness, solidity, and persistence.

The parasitism of *Boletus parasiticus* on *Scleroderma vulgare* and *S. verrucosum* finds a parallel instance among the Ascomycetes in the parasitism of *Cordyceps ophioglossoides* and *C. capitata* upon *Elaphomyces granulatus*, *E. variegatus*, and *E. muricatus*, with this difference: that, while the fruit-bodies of the Sclerodermas are subaerial, those of the Elaphomyces species are subterranean. *Scleroderma vulgare*[3]

[1] M. J. Berkeley, *Outlines of Fungology*, London, 1860, Plate XV, Fig. 4.

[2] E. Boudier, *Icones Fungorum*, Paris, 1904–1911, Tab. CLXV.

[3] W. B. McDougall, "On the Mycorrhizas of Forest Trees," *American Journal of Botany*, vol. i, 1914, pp. 51–74.

and *Elaphomyces granulatus*[1] form mycorrhizae with the roots of trees. There can be no doubt that *E. granulatus* is dependent for its nutriment on the Pines, etc., with which it is associated and, in all probability, *S. vulgare* is dependent on Oaks, etc. Granted that *S. vulgare* and *E. granulatus* are both more or less parasitic on the roots of trees, we are presented with two interesting chains of parasitism in the web of life. On the one hand, a living Oak tree may support a living *Scleroderma vulgare* and this in turn may support a living *Boletus parasiticus*, the *B. parasiticus* plant thus being dependent indirectly on the Oak ; and, on the other hand, a living Pine tree may support a living *Elaphomyces granulatus*, and this in turn may support a living *Cordyceps ophioglossoides*, the *C. ophioglossoides* plant thus being dependent indirectly on the Pine.

FIG. 183.—*Leptonia parasitica*, as a parasite producing fruit-bodies on the under side of the pileus of *Cantharellus cibarius*. Spores to the left. Quélet's illustration, copied by Dr. Nellie Carter. The fruit-body, natural size.

Leptonia parasitica.—*Leptonia parasitica* (Fig. 183) is a small pink-spored lamellate agaric, with a short stipe and a pileus 5–7 mm. wide, which has been found in France growing on the under side of the pileus of *Cantharellus cibarius*. It was described and illustrated by Quélet[2] in 1878. In England it has never yet been recorded.

Quélet's description of *Leptonia parasitica*, translated from the French, is as follows : "Finely tomentose-pubescent and snow-white. Stipe slender, arcuate, *dilated above*. Pileus membranaceous, translucent, convex-plane (5–7 mm.), somewhat umbilicate. Gills sinuate, ventricose, white then pink. Spores pentagonal (12 μ), guttulate and pink. In summer, on *Cantharellus cibarius* which its mycelium covers with a delicate arachnoid network."

Claudopus subdepluens.—*Claudopus subdepluens* was discovered

[1] M. Reess, "Ueber den Parasitismus von *Elaphomyces granulatus*," *Sitzungsberichte der phys.-med. Soc. zu Erlangen*, Mai, 1880, pp. 729-733. Also M. Reess und C. Fisch, "Untersuchungen über Bau und Lebensgeschichte der Hirschtrüffel, Elaphomyces," *Bibliotheca botanica*, Bd. II, Heft 7, 1887, pp. 1–26, Taf. I.

[2] L. Quélet, "Quelques espèces nouvelles de champignons," *Bull. Soc. Bot. France*, T. XXV, 1878, p. 287, Plate III, Fig. 6.

by Fitzpatrick [1] in August, 1914, at Six Mile Gorge, near Ithaca, in the State of New York, growing on *Polyporus perennis*. It is the only species of Agaricineae known to be parasitic on one of the Polyporeae.

Fitzpatrick remarks that, at Six Mile Gorge, there were numerous fruit-bodies of *Polyporus perennis* but only a few which were parasitised. Among his other observations were the following :

The parasitised fruit-bodies of *Polyporus perennis* give but little evidence of the presence of the parasite, for they are normal in their general appearance, show no trace of either hypertrophy or dwarfing and, when seen from above, cannot be distinguished from fruit-bodies which are unparasitised. The fruit-bodies of the parasite, *Claudopus subdepluens*, are minute and occur in considerable numbers about the mouths of the hymenial tubes and along the stipe of their host-plant. An examination with hand-lens reveals that certain of the hymenial tubes of the Polyporus in the immediate vicinity of the fruit-bodies of the parasite are partially filled with the greyish mycelium of the Claudopus.

In Fig. 184, from below and twice the natural size, are to be seen three or four fused pilei of *Polyporus perennis* [2] bearing over one hundred fruit-bodies of the Claudopus. Of the Claudopus fruit-bodies only about half a dozen are fully expanded, while the rest are in various stages of development. The blurs in the photograph are the stipes of the host fruit-bodies, which failed to come into focus. In Fig. 185 the expanded fruit-bodies of Fig. 184 are shown four times the natural size.

The fruit-body of *Claudopus subdepluens* is described as follows. Pileus at first convex, then expanded, minute, maximum diameter 1·4 mm., white, minutely tomentose, margin sulcate ; gills salmon-coloured from the first, distant, adnate, edge quite entire ; stipe white, lateral, curved, up to 2 mm. long, less than 0·5 mm. thick ; basidia clavate, provided with 4 sterigmata ; spores angular,

[1] H. M. Fitzpatrick, "A Parasitic Species of Claudopus," *Mycologia*, vol. vii, 1915, pp. 34–37.

[2] When the fruit-bodies of *Polyporus perennis* grow very near to one another, the pilei frequently become confluent. *Cf.* these *Researches*, vol. ii, 1922, p. 81.

FIG. 184.—*Claudopus subdepluens* Fitzpatrick (? = *Leptonia parasitica* Quélet), growing as a parasite on *Polyporus perennis*. The minute fruit-bodies of the Claudopus are to be seen in various stages of development attached to the hymenial tubes on the under side of the pileus of the host-plant. The pilei of three or four fruit-bodies of *P. perennis* are here fused together. The blurs are due to the presence of the stipes which were out of focus. Found at Six Mile Gorge, near Ithaca, New York State, and photographed by H. M. Fitzpatrick. Twice the natural size.

uniguttulate (rarely with two or more guttules), faintly but distinctly pink, 7–12 × 6–8 μ.[1]

To the parasite on *Polyporus perennis* Fitzpatrick gave the name

[1] H. M. Fitzpatrick, *loc. cit.*, p. 37. The original description is given in Latin.

Claudopus subdepluens because he considered that it is closely related to *C. depluens* described by Peck.[1] The fruit-bodies of the latter species, however, are distinguished from those of the former by their much larger size, their lamellae being at first white, and by arising not on another agaric but on the ground or decaying wood from a saprophytic mycelium. Recently, Fitzpatrick has expressed the view that *Claudopus subdepluens* may be identical with Quélet's *Leptonia parasitica*.[2]

Fitzpatrick has attempted to solve the problem of the relation of *Claudopus subdepluens* with its host *Polyporus perennis*. After remarking that the only external evidence of a diseased condition in the infected Polyporus is the presence of Claudopus mycelium partially filling certain of the hymenial tubes close by the Claudopus fruit-bodies, he continues as follows: "The sporophores of the Polyporus produce their hymenium in the normal manner. Thin sections made through the point of attachment of the stipe of the parasite to the host disclose no marked derangement of the elements of the latter. It is possible to trace to some extent the course of the hyaline hyphae of the Claudopus among the deeper-coloured threads making up the sporophore of the Polyporus. Some of these are found ramifying on the trama of the host to a considerable depth. It is possible that they extend through its stipe to the soil. The presence of fruit-bodies of the parasite on the stipe furnishes some indication of this.[3]

"The mycelium of the parasite is relatively small in amount, and the hyphae of the two fungi lie in close contact and run approximately parallel. Careful search fails to reveal any organs of the nature of haustoria, and dissolution of the host hyphae by enzymes excreted by the parasite appears not to take place. If any such process occurs, the disintegration of the host is insufficient in amount to be evident in thin, free-hand sections.[4]

[1] C. H. Peck, "New York species of Pleurotus, Claudopus, and Crepidotus," *Annual Report N.Y. State Museum*, vol. xxxix, 1887, p. 68.

[2] *In litt.* Dr. Fitzpatrick informs me, however, that, since the stipes of his fruit-bodies were distinctly lateral, he thinks the species must be included in *Claudopus* and not in *Leptonia*. Whether or not the two species are identical seems to me very uncertain. I have therefore thought it best to treat them as distinct.

[3] H. M. Fitzpatrick, *loc. cit.*, p. 35. [4] *Ibid.*

"The method by which natural infection occurs was not determined. Fruit-bodies of the Claudopus leading a saprophytic existence on neighbouring twigs or soil could not be found. It is not impossible, however, that these were produced earlier and had already disappeared. Local infection of the sporophores of the Polyporus might result either from spores produced on such sapro-

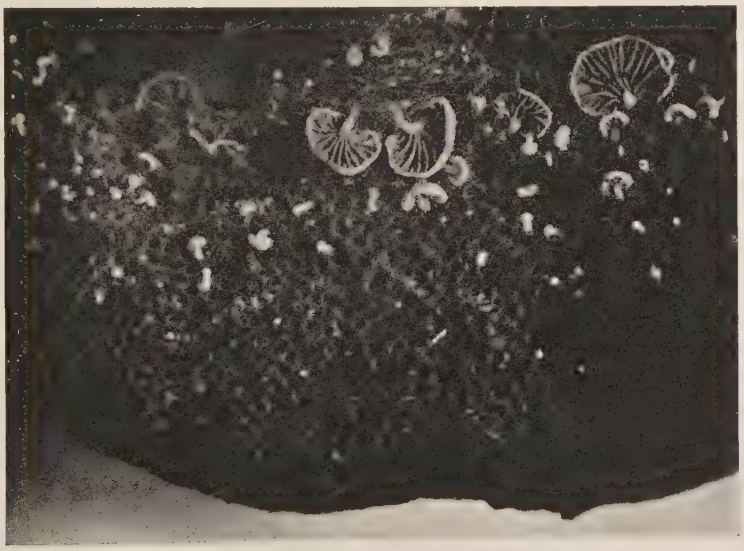


Fig. 185.—*Claudopus subdepluens* Fitzpatrick (? = *Leptonia parasitica* Quélet) growing as a parasite on *Polyporus perennis*. Some of Claudopus fruit-bodies of Fig. 184 shown four times the natural size. The lateral position of the stipe and the distant gills can be clearly perceived. Found at Six Mile Gorge, near Ithaca, New York State, and photographed by H. M. Fitzpatrick.

phytic fruit-bodies or from hibernated spores produced the preceding year on parasitic fruit-bodies. It seems more probable, however, that the vegetative mycelium of the *Claudopus* spreads in the soil and travels upward among the hyphae of the Polyporus during the development of its sporophore. It may thus retain organic connection with the food material in the soil and be partially or wholly independent of the Polyporus in its food relationships." [1]

From the above discussion by Fitzpatrick it will be seen that it is as yet uncertain whether *Claudopus subdepluens* derives its

[1] H. M. Fitzpatrick, *loc. cit.*, p. 35.

nutriment from *Polyporus perennis* fruit-bodies or merely uses the Polyporus fruit-bodies as an apparatus to which it may attach its own fruit-bodies. An experimental investigation alone can decide between these two alternatives; but, basing my opinion on our knowledge of actual parasitism in the case of *Stropharia epimyces* on *Coprinus comatus* and *C. atramentarius*, of *Boletus parasiticus* on *Scleroderma vulgare* and *S. verrucosum*, and of *Nyctalis asterophora* and *N. parasitica* on certain Russulae, I am inclined to the first alternative, namely, that *Claudopus subdepluens*, when found on *Polyporus perennis*, is truly parasitic and depends on the Polyporus for its food-supply. It is of course possible that *C. subdepluens* normally lives a saprophytic existence on wood, etc., and is only occasionally a parasite on *Polyporus perennis*.

Volvaria Loveiana.—*Volvaria Loveiana* is a rare pink-spored volvate agaric which is occasionally found parasitic on the large and substantial fruit-bodies of *Clitocybe nebularis*, and sometimes also on *C. clavipes*.[1] It occurs in England, France, Germany, Canada (Ontario), and the United States of North America (Minnesota).[2]

The first record of *Volvaria Loveiana* is in Knapp's *Journal of a Naturalist*[3] where, in a wood-cut, two specimens are shown, each seated on the top of a *Clitocybe nebularis*. Knapp called the fungus *Agaricus surrectus* and remarks: "We have seen an agaric with a bulbous root and downy pileus that will spring from the smooth summit of another which has a uniform foot-stalk, though not of common occurrence. Thus a plant that arises from decay is found to constitute a soil for another, and the termination of the chain of efficiency is hidden from us."

Volvaria Loveiana was first carefully described by Berkeley[4]

[1] This second host is mentioned by René Maire (*vide infra*, "Notes critiques, etc.," p. 422) and by A. Ricken (*Die Blätterpilze*, Leipzig, 1915, p. 275).

[2] E. T. Harper, "Two Parasitic Mushrooms," *Mycologia*, vol. viii, 1916, p. 65. Dearness collected specimens at London, Ontario, in 1896. *Ibid.*, p. 68.

[3] J. L. Knapp, *The Journal of a Naturalist*, London, ed. ii, 1829, pp. 377–379.

[4] M. J. Berkeley, in W. J. Hooker's *English Flora*, vol. v, part ii, London, 1836, pp. 104–105. Berkeley was evidently not aware that the fungus had been recorded by Knapp, for he refers to it as "a most elegant and curious species which . . . appears not to have been hitherto noticed." The illustration shown in Fig. 186, taken from Berkeley's *Outlines of Fungology*, London, 1860, Plate VII, Fig. 2, has been enlarged to natural size.

who gave it the specific name *Loveiana* as a tribute to the Rev. R. T. Lowe ; and Berkeley's illustration showing its association with *Clitocybe nebularis* is reproduced in Fig. 186. Photographs of the parasite upon its host have been published by Maire[1] and Harper,[2] and coloured illustrations by Berkeley,[3] Cooke,[4] and Gillet.[5]

It seems very likely that *Volvaria Loveiana* can live either as a saprophyte or as a parasite. Quélet [6] considered that *V. Loveiana* is a luxuriant parasitic form of *V. plumosa* Lasch, a fungus which grows on the ground among humus in fir woods. Maire [7] studied both species and has come to the conclusion that they are identical. Harper,[8] who is inclined to agree with Maire, says : " The plants are certainly closely related, though cultures would be necessary to prove their identity."

Berkeley's illustration of *Volvaria Loveiana* shows a host Clitocybe which has a normal appearance, but Maire remarks that the host plants are often more or less deformed, while Harper found host plants (*Clitocybe nebularis*) which had become reduced to irregular masses, reminding one of *Coprinus comatus* when attacked by *Stropharia epimyces*. A host fruit-body which is not at all, or only slightly, deformed probably succeeds in liberating a certain number of spores, but one which is badly deformed may well be completely sterile.

The following is a description of *Volvaria Loveiana* as given by Rea,[9] who saw the fungus in England : " Pileus 5–7 cm., *white, with a very slight shade of pink*, or cinereous, subtruncato-globose, then convex, or slightly expanded, *beautifully silky* ; margin involute. Stipe $5 \times 0{\cdot}5$–1 cm., *pure white*, bulbose, attenuated upwards, *closely fibrillose, with a little matted down*, very juicy.

[1] René Maire, " Notes critiques sur quelques champignons," *Bull. Soc. Myc. Fr.*, T. XXVII, 1911, Plate XIII, Fig. 1.

[2] E. T. Harper, *loc. cit.*, Plate 177, A.

[3] M. J. Berkeley, *loc. cit.*

[4] M. C. Cooke, *Illustrations of British Fungi*, Plate 295.

[5] G.-G. Gillet, *Les Hyménomycètes*, Alençon, 1874, p. 387, Plate 797.

[6] L. Quélet, *vide* René Maire, *loc. cit.*, p. 420.

[7] René Maire, *loc. cit.*, pp. 420–423. Maire shows photographs of the two species in the same Plate.

[8] E. T. Harper, *loc. cit.*, pp. 66–67.

[9] Carleton Rea, *British Basidiomycetae*, Cambridge, 1922, pp. 94–95.

Volva *pure white*, with a little downy prominence within round the base of the stem. Gills *white, becoming gradually pale pink*, free,

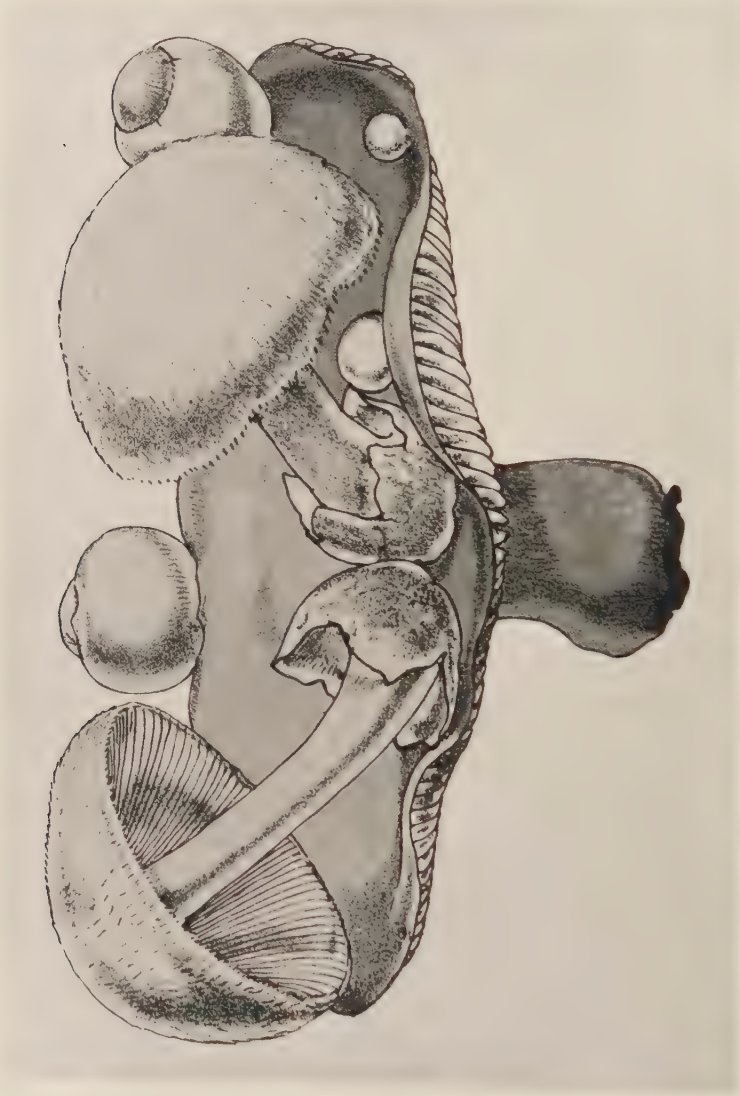


Fig. 186.—*Volvaria Loveiana* as a parasite on *Clitocybe nebularis*. From M. J. Berkeley's *Outlines of British Fungology*. Natural size.

broad in front, subdeliquescent. Flesh *white, becoming yellowish*. Spores pink, elliptical, 5–6 × 3–4 μ. 1-guttulate. On *Clitocybe nebularis* (Batsch) Fr. October. Rare."

In 1867 W. G. Smith [1] published a brief communication on *Volvaria Loveiana* in which he stated that he had successfully cultivated this parasite on its host-plant. He says: *Clitocybe nebularis* "takes its name from its singular cinereous *clouded* pileus, which is generally slate-coloured, deep grey, or brown, *clouded with white*. This white tint is sometimes a mere bloom, at other times (and generally in old and distorted specimens) it acquires the character of a thick floccose web, attaining a thickness of a sixteenth of an inch.

"Last summer the thought struck me that the white substance so common on *Agaricus nebularis* might be nothing less than a mere state of the mycelium of *Agaricus Loveianus* itself, and only requiring certain conditions to enable it to develop into the perfect plant . . . sent me a batch of Fungi found growing among rotten fir leaves. . . . After gathering all the rotten fir-leaves, and strongly saturating them with rain-water, I half-buried the plants of *A. nebularis* among them, and placed all together under a bell-glass in a warm room. The white substance then soon showed its true character, and ran over the whole mass, making no distinction of either pileus, stipe, or gills; small white nodosities soon began to appear, and after a fortnight I had the pleasure of seeing the fully-developed *A. Loveianus*. My specimens were small, the volvas very large, and pale sienna in colour. . . . Not the least singular part of the case is, that though I had repeatedly written . . . regarding this agaric, with request for a sharp look out for it, nothing has ever been seen of it in the fir plantations."

Mr. J. Ramsbottom,[2] who read Smith's paper about twelve years ago, has informed me that, after examining numerous pilei of *Clitocybe nebularis*, he has never been able to find any evidence that the cloudiness is due to a foreign mycelium, and that he has several times attempted to repeat Smith's experiment, but never with any success.

Doubtless the pileus of *Clitocybe nebularis* exhibits two kinds of cloudiness: (1) a normal one, due to its own hyphae,—a mere

[1] W. G. Smith, "On the Artificial Production of *Agaricus* (*Volvaria*) *Loveianus* Berk.," *Journ. of Bot.*, vol. v, 1867, pp. 365–367.

[2] *In litt.*

bloom, and (2) an abnormal one, to be observed only occasionally, due to the presence of the floccose white mycelium of *Volvaria Loveiana*. This second kind of cloudiness, which was seen by Smith, appears to be rare ; but only from such pilei as show it could one hope to raise fruit-bodies of *V. Loveiana*.

It is very desirable that some one should attempt to produce fruit-bodies of *V. Loveiana* by sowing the spores of *V. plumosa* (which Quélet and Maire consider to be identical with *V. Loveiana*) on a healthy pileus of *Clitocybe nebularis*. If such an experiment should be crowned with success, excellent material might be obtained for working out the details of the relation between the parasite and its host.

The Genus Nyctalis. — The genus Nyctalis is believed to be closely related to Cantharellus. It resembles Cantharellus in having fleshy white-spored fruit-bodies in which the hymenium is spread over shallow obtuse gills, and in which the stipe and pileus are confluent ; but it differs from Cantharellus in its gills being simple instead of forked. Moreover, while all the species of Cantharellus are saprophytes, some of those of Nyctalis are highly specialised parasites.

The best known species of Nyctalis are *N. asterophora* and *N. parasitica* which are parasitic on certain other agarics ; and it is to the first of these that we shall now turn our attention.

Nyctalis asterophora.—*Nyctalis asterophora* (Fig. 187) occurs in Europe, North America,[1] and Japan [2] as a parasite on certain large and fleshy agarics. It was discovered by Micheli [3] in 1729 on *Lactarius piperatus*, and first carefully illustrated by Bulliard [4] who shows it growing from the upper surface of the pileus and from the gills of *Collybia fusipes*. De Bary [5] found it on *Russula nigricans*

[1] C. H. Kauffman, *The Agaricaceae of Michigan, U.S.A.*, 1918, vol. i, p. 32 ; vol. ii, Plate I.

[2] T. Iwasaki, *Honzō-zufu* (Botanical Illustrations), Tokio, 1829, Plate 33. A copy of this rare work is in the library of Kew Gardens. The author states (in Japanese) that the powder on the pileus resembles that of Puff-balls.

[3] P. A. Micheli, *Nova Plantarum Genera*, Florentiae, 1729, p. 200, Tab. 82, Fig. 1.

[4] P. Bulliard, *Herbier de la France*, Paris, 1784, Plate 166. Bulliard says that he found the parasite always on *l'Agaric pied-fu* and his excellent illustration leaves no doubt that this fungus is *Collybia fusipes*.

[5] A. de Bary, "Zur Kenntniss einiger Agaricinen," *Bot. Zeit.*, Bd. XVII, 1859, p. 385.

and *R. adusta*, and Ricken[1] records it as occurring on *Lactarius vellereus*. It appears to be most common on *Russula nigricans*. All its hosts agree in being large, fleshy, and somewhat persistent. It is thus described by Rea :[2]

" Pileus 1–1·5 cm., *white*, then *fawn colour from the large stellate chlamydospores*, 15–20 μ, fleshy, conical, then hemispherical, *floccoso-pulverulent*. Stipe 1–2 cm. × 2–4 mm., *white, then fuscous*, equal, often twisted, pruinose. Gills *white, then dingy*, adnate, distant, thick, tense and straight, somewhat forked, *often wanting*. Flesh *dark grey*. Spores *white*, ' elliptical, 6 × 4 μ ' (Ricken). On dead *Russula nigricans*. July–November. Common."

A photograph showing *N. asterophora* on its host is reproduced in Fig. 187. An excellent coloured drawing is given by Brefeld,[3] and other illustrations by Cooke[4] and Kauffman.[5]

The rather fleshy hemispherical pileus of *Nyctalis asterophora* is characterised by two special features : (1) the poor development of the gills, these being often either almost or entirely wanting (Fig. 188, A, B, C, D, E, H), and (2) the breaking up of the pileus-flesh into a pulverulent fawn-coloured stratum containing a vast number of stellate chlamydospores (Fig. 188, B, D, E, F, G).

The shallow obtuse gills of *Nyctalis asterophora*, as just pointed out, may be altogether suppressed ; but, even when present, they by no means always produce basidiospores. Brefeld[6] states that a normal hymenium bearing basidiospores is only present on the gills of the largest fruit-bodies, while de Bary[7] remarks that, even when fruit-bodies are fertile, the number of basidiospores produced is relatively small (Fig. 188, H, I). Moreover, when ripe, the chlamydospores are carried off by the wind like the spores of Smut Fungi or Puff-balls. These facts suggest that *N. parasitica* reproduces itself, not like most other Agaricineae by basidiospores, but by means of its chlamydospores.

[1] A. Ricken, *Die Blätterpilze*, Leipzig, 1915, p. 8.

[2] C. Rea, *British Basidiomycetae*, Cambridge, 1922, p. 541.

[3] O. Brefeld, *Untersuchungen über Pilze*, Leipzig, Heft VIII, 1889, Tab. V., Fig. I.

[4] M. C. Cooke, *Illustrations of British Fungi*, Plate 1132.

[5] C. H. Kauffman, *loc. cit.*

[6] O. Brefeld, *loc. cit.*, p. 79.

[7] A. de Bary, *loc. cit.*, p. 388.

That the stellate chlamydospores of *Nyctalis asterophora* are able to germinate and bring about the reproduction of the fungus was proved nearly one hundred years ago (1831) by Krombholz,[1] who sowed some chlamydospores on the pileus of a healthy *Russula*

FIG. 187.—*Nyctalis asterophora* growing as a parasite on *Russula nigricans*. The host fruit-body owes its blackness to the presence of dark pigments produced by the action of oxydases. The pilei of the younger fruit-bodies of the Nyctalis are still white and fleshy ; but those of the two oldest and largest are fawn-coloured and floccoso-pulverulent, owing to the presence of great numbers of fawn-coloured powdery chlamydospores. The mature pilei of *N. parasitica*, as may be seen here, are hemispherical in shape. Photographed at Cornell University, Ithaca, New York State, by H. M. Fitzpatrick. Natural size.

adusta and thereby obtained fruit-bodies of *Nyctalis asterophora* within three weeks.[2] Fifty-eight years later (1889), this remarkable

[1] J. V. Krombholz, *Naturgetreue Abbildungen und Beschreibungen der essbaren, schädlichen und verdächtigen Schwämme*, Prag, Heft 1, 1831, pp. 5–6.

[2] The experiment of Krombholz, from the point of view of the phytopathologist, is of peculiar historical interest, in that it is one of the first experiments on record in which an attempt was made to propagate a parasite by sowing its spores on the host-plant. However, it cannot be described as the very first inoculation experiment; for Schöler, in 1816, and Bönninghausen, in 1818, both succeeded in infecting Rye plants by rubbing on their laminae some Barberry leaves which were spotted with the accidia of *Puccinia graminis*, the well-known Black Stem Rust Fungus. *Vide* (1) N. P. Schöler, "En Afhandling om Berberissens skadelige Virkning paa Saeden," *Land-oekonom. Tidende*, vol. viii, 1818; and (2) C. von Bönninghausen, *Möglin's Annalen der Landwirtschaft*, Bd. IV, 1818, p. 280.

experiment was repeated successfully by Brefeld.[1] The account which Krombholz gives of his observations, translated from the German, is as follows:

"Some fresh spore-dust from an *Asterophora lycoperdoides* (= *Nyctalis asterophora*) fungus, which was fully developed and had already lost its pellicle, was placed on a young perfectly healthy Kohlenstäubling (*Agaricus adustus*) which had been carefully washed with spring water. Several other Kohlenstäublings taken from the same group of fungi, some of which were cleaned and others not, were then placed along with the inoculated specimen under a bell-jar and left in my work-room in moderate light. On the first day (24 hours after sowing the spores) no change could be observed. After 48 hours, one could see that the still fresh-looking fungus was sweating out some fluid at those places where the spores had been sown. On the third day the little heaps of brown spores had become somewhat pale and covered with a felt-work of fine white threads, which were at first simple and afterwards branched, and which could be detected only with the help of a very strong lens. After from three to four days, the lens revealed a few white points. On the fifth day these had attained a height of $\frac{1}{6}$ to $\frac{1}{4}$ of a line and had taken on a cylindrical form. During the sixth, seventh, and eighth days, owing to the increased destruction of the Kohlenstäubling, some of them pushed up farther and farther. They attained the height of a line, their upper ends became somewhat swollen and, on the ninth day, most of them had a height of $1\frac{1}{2}$–2 lines and upwards; their caps were spherical and floccose, their stalks round and completely formed.

"Up to now the parasitic fungi were visible only just at those precisely marked spots where the spores had been sown, and at no other place was there any similar development. In the meanwhile most of the unsown specimens of the Kohlenstäubling had gone rotten (zerflossen); and, under the same conditions of temperature, light, moisture, and air, they yielded not a trace of any fungus-production, although they had been taken from the same group of fungi as those specimens upon which the spores had been deposited. The inoculated, Asterophora-bearing fungi lived longer and broke

[1] O. Brefeld, *Untersuchungen über Pilze*, Leipzig, Heft VIII, 1889, p. 70 *et seq.*

FIG. 188.—*Nyctalis asterophora*. A, a young and partially developed fruit-body with a small rounded pileus and no hymenium (= *Asterophora trichioides* Fr.). Natural size. B, longitudinal section through the upper part of a fruit-body somewhat further developed, showing numerous chlamydospores in the outer stratum of the pileus-flesh; hymenium and gills wanting. Magnification, 90. C, longitudinal section through a larger fruit-body, seen by reflected light. Natural size. D, the same section as C, magnified about 4 times, seen by transmitted light: *a*, chlamydospore-layer; *b*, sterile layer (hymenophorum); *h*, hymenium; gills absent. E, a longitudinal section through a fully developed fruit-body with definite gills, seen by reflected light; the white outer stratum of the pileus-flesh is the chlamydospore-layer. Natural size. F, a ripe chlamydospore from the chlamydospore-layer of a pileus. G: *a*, a hypha about to form a chlamydospore; *b*, a ripe chlamydospore. F and G, magnification, 390. H, transverse section through one of the gills of E; *h*, the hymenium with a few of the basidia bearing basidiospores. Magnification, 120. I, hyphae removed from the hymenium: *a*, basidia with some of the subhymenial cells; *b*, a basidium bearing spores. Magnification, 390. The drawings copied by Dr. Nellie Carter from de Bary's paper in the *Botanische Zeitung* (Bd. XVII, 1859) and arranged by the author.

down with difficulty ; their substance remained firm, and they conducted all their juices to the parasite, the neighbourhood of which was continually moist. From the tenth to the fourteenth day the fungi (*Asterophora lycoperdoides*) grew quickly and at the end they were already four lines high. The caps were smooth and the stalks floccose, but all as yet colourless.

" On the fifteenth day one or two fungi of a group took the lead and grew apparently at the cost of the others.

" On the twentieth day the fibrillae had already disappeared from the stalk which had become smooth and had attained a height of $\frac{1}{2}$–$1\frac{1}{2}$ inches.

" However, the cap became floccose, and its margin separated itself from the surface of the stalk and became floccose ; but the individual lamellae were not yet visible. At this stage the growth of the stalk or stipe appeared to have come to an end and the development of the cap began to go forward so much the faster.

" The cap, which earlier had been rounded, elongated, or spherical, now became hemispherical, which form, however, with the appearance of the lamellae, changed to that of a cushion. This was accompanied by the straight stalk becoming shiny and smooth and the cap breaking up in a floccose manner. Through the breaking up of the cap into flocculi there became visible the included spores (chlamydospores) which bulged out as a mass of powder.

" The youngest scarcely established fungus-balls three or four days old, as also the pellicles of the caps of the somewhat older fungi, enclosed from the twelfth to the fourteenth day a fleshy, textureless, more or less firm mass of tissue. On the seventeenth or eighteenth day this substance received much sap and, on the eighteenth day, became watery. On the very same day it became firmer again, on the nineteenth day lost almost all its fluidity and, on the twentieth to the twenty-first day, was again dry and powdery. While solidification was in progress, it was already possible to find a few star-like seeds (chlamydospores) intermixed with hyphae and dried, but from the twenty-first to the twenty-third day all the spores were regularly star-shaped.

" These fungi produced by sowing spores were very different in appearance from the mother-plant.

"The mother-plant had a height of 3–4½ inches and was completely white; its stalk was bent; and its lamellae, as also the edge of the pileus, were white. The fungi obtained through sowing spores, on the other hand, were small, ½–1½ inches high; their stalks were perfectly upright and grey; their lamellae were grey-blue; and the cap had no margin. They looked exactly like the fungi illustrated by Ditmar (Sturm: *Deutschl. Flora*, III Abth., 1 Bändch.)."

Krombholz's experiment is highly instructive, for it affords strong evidence that *Nyctalis asterophora*: (1) can attack a living fruit-body of *Russula nigricans* and is therefore a true parasite, (2) can enter the host fruit-body very readily *via* the pellicle covering the pileus-flesh, (3) can reproduce itself by means of its chlamydospores, and (4) can complete its development from chlamydospore to chlamydospore within about three weeks.

Until the time of Brefeld, but little notice was taken of Krombholz's experiment upon the propagation of *Nyctalis asterophora* and, in the meanwhile, there grew up an erroneous conception of the nature of the chlamydospores. Corda [1] and Bonorden [2] regarded the connection of the chlamydospores with the Nyctalis as unproved and favoured the view that the chlamydospores belong to a fungus that is parasitic on the Nyctalis. Tulasne [3] then expressed this view more distinctly and stated that the chlamydospores of *Nyctalis asterophora* and *N. parasitica* belong not to these two species of Nyctalis, but to two parasitic fungi which he included in the sphaeriaceous genus Hypomyces. The supposed parasite on *Nyctalis asterophora* he called *Hypomyces asterophorus*, and the supposed parasite on *N. parasitica H. Baryanus*. According to Tulasne, these two species of Hypomyces live parasitically on a single species of Nyctalis [4] in such a manner that, when the Nyctalis is attacked by *H. asterophorus*, it becomes transformed into *N. asterophora*

[1] A. C. I. Corda, *Icones Fungorum hucusque cognitorum*, Prag, T. IV, 1840, p. 8.

[2] H. F. Bonorden, *Handbuch der allgemeinen Mycologie*, Stuttgart, 1851, p. 82.

[3] L. R. Tulasne, "De quelques Sphéries fongicoles à propos d'un mémoire de M. Antoine de Bary sur les Nyctalis," *Ann sci. nat.*, 4 sér., T. XIII, 1860, pp. 5–15. Also *Selecta Fungorum Carpologia*, T. III, 1865, pp. 54–59.

[4] Tulasne refers to this agaric as *Agaricus parasiticus* Bull., "De quelques Sphéries, etc.," pp. 13–14, etc.

and, when attacked by *H. Baryanus*, it becomes transformed into *N. parasitica*. Tulasne remarked upon the similarity in appearance of the chlamydospores of Nyctalis and the chlamydospores of Hypomyces species which are known to be parasitic on agarics and, in a Plate of illustrations, he shows the chlamydospores of Nyctalis along with the perithecia of a Hypomyces.[1] However, he expressly pointed out that he had not been able to trace the direct and untrammelled continuation of the two structures in one and the same mycelium.

In 1859 de Bary [2] investigated *Nyctalis asterophora* and *N. parasitica* anatomically and came to the conclusion that the two forms of chlamydospores developed on the pilei of these species are produced by hyphae which are branches of the pilear hyphae and, therefore, clearly belong to the two agarics in which they are found. The account of Tulasne's investigation on Nyctalis was published a little later than de Bary's. De Bary, finding himself in opposition to so able a mycologist as Tulasne, repeated his observations. However, he again saw that the hyphae which bear the chlamydospores are continuous with the hyphae of the Nyctalis pilei, and he was therefore unable to alter his opinion as previously expressed. In 1884 he [3] criticised Tulasne's Hypomyces hypothesis as follows : " If one holds simply to the facts as they present themselves, it is necessary to retain my earlier view, the more so as an *Agaricus parasiticus* without one or other of the chlamydospore structures, so far as I know, is entirely unknown. Also, seeing that the structure of the fruit-body of *Nyctalis parasitica* is so very different from that of *N. asterophora*, Tulasne's hypothesis that both these species are forms of the same *Agaricus parasiticus*, which has suffered a different mode of degeneration through the action of the two different parasites *Hypomyces parasiticus* and *H. asterophorus*, becomes far more doubtful of belief than my above expressed opinion."

In 1889 the whole question of the status of the chlamydospores in the two species of Nyctalis was settled once and for all by means

[1] L. R. Tulasne, *Selecta Fungorum Carpologia*, T. III, 1865, Tab. IX.

[2] A. de Bary, " Zur Kenntniss einiger Agaricinen," *Bot. Zeit.*, Bd. XVII, 1859, pp. 385–388, 393–398.

[3] A. de Bary, *Vergleichende Morph. u. Biol. der Pilze*, Leipzig, 1884, pp. 361–362.

of a brilliant piece of cultural work carried out by Brefeld.[1] Brefeld not only repeated the experiment of Krombholz, *i.e.* sowed chlamydospores of *Nyctalis asterophora* on the pileus of a Russula and obtained fruit-bodies of *N. asterophora* as a result, but he traced the life-history of *N. asterophora* by means of pure cultures.[2] His observations [3] on *N. asterophora* were as follows :

So far as basidiospores are concerned, most of the fruit-bodies of *Nyctalis asterophora* are completely sterile, for fertile basidia are only found exceptionally on fruit-bodies which are large and vigorous. If freshly deposited basidiospores are sown in a decoction of Russula fruit-bodies, they germinate readily and produce small mycelia. In each mycelium some of the branches soon begin to break up into chains of oidia (Fig. 189, nos. 2 and 3) resembling those of *Endomyces decipiens* among the Ascomycetes and of many Hydneae and Polyporeae among the Autobasidiomycetes. The oidia in their turn germinate readily and produce mycelia which again break up into oidia. In a series of generations produced by oidia oidia-formation becomes less and less pronounced and more and more replaced by the vegetative growth of the hyphae. At the same time typical stellate chlamydospores, just like those occurring in the upper stratum of a pileus, begin to be formed in the hyphae, at first along with oidia, but subsequently alone (Fig. 189, nos. 1 and 4). The hyphae which give rise to chlamydospores are all made up of cells which are separated from one another by walls bearing clamp-connections. The chlamydospores are constructed at the end of the hypha or along its course, and chains of chlamydo-

[1] O. Brefeld, "Die Gattung Nyctalis," *Untersuchungen über Pilze*, Leipzig, Heft VIII, p. 70 *et seq.*

[2] Any one who desires to gain an insight into Brefeld's methods of research and the extraordinary skill with which he was able to bring to a successful conclusion a difficult piece of experimental work, could not do better than read carefully the account of his researches on the life-history of *Nyctalis asterophora* and *N. parasitica*. His solution of the chlamydospore question was a veritable triumph, and one can readily understand the satisfaction and pleasure with which he must have written down his results and presented them to the botanical world. If Brefeld had done no more than provide us with his account of Nyctalis, he would have done enough to compel us to regard him as one of the ablest botanists of the nineteenth century.

[3] These observations are well summarised by F. von Tavel in his *Vergleichende Morphologie der Pilze*, Jena, 1892, pp. 168–170.

spores may thus come into existence. A cell about to produce a chlamydospore becomes swollen in the middle, the cell-contents then collect in the swelling, and a cross-wall is formed at each end of the swelling. Thus the cell becomes divided into three cells, a large central chlamydospore in course of development and two small empty end-cells. The contents of the chlamydospore now contract somewhat and become surrounded by a new wall. Next the wall bulges forward in certain places and thus the chlamydospore comes to take on its star-like shape. The wall now thickens and turns brown. Finally, owing to the swelling of the walls of the empty end-cells between adjacent chlamydospores, the individual chlamydospores become free. This mode of formation of the chlamydospores of *Nyctalis asterophora* is essentially similar to that of the chlamydospores of Ptychogaster and Fistulina. The germination of the chlamydospores takes place with difficulty, for it occurs only on the surface of a Russula fruit-body. The production of the chlamydospores is extremely rich and long-continued and, frequently, whole mycelia become converted into them.

Only seldom in a mycelium under cultivation do certain parts grow more vigorously. In these parts the fruit-bodies of the Nyctalis arise by the growth of hyphae which form a knot and then develop in the same manner as the fruit-bodies of other Agaricineae having a rudimentary volva. The snow-white pileus begins to turn brown when the stipe elongates. Then its hyphae form chlamydospores in the same manner as the mycelium, except for the fact that chlamydospore-formation is restricted to the upper part of the pileus where the chlamydospores become heaped up in thick layers as a brown powder. Most of the nutrient substance at the disposal of the fungus becomes used up during this fructification, and when chlamydospore-formation begins, growth in size comes to an end. The hymenium, as a rule, becomes aborted, and only in large fruit-bodies in which chlamydospores are formed late do the basidia produce basidiospores. Thus in *Nyctalis asterophora*, as regards chlamydospores and basidiospores, we have similar conditions to those which prevail in *Ptychogaster albus*.[1]

Brefeld's investigation of *Nyctalis asterophora*, so admirably

[1] For an account of *Ptychogaster albus*, *vide infra*, vol. iv.

carried out, not only shows that the life-history of this fungus can be followed step by step in pure cultures, but proves conclusively :

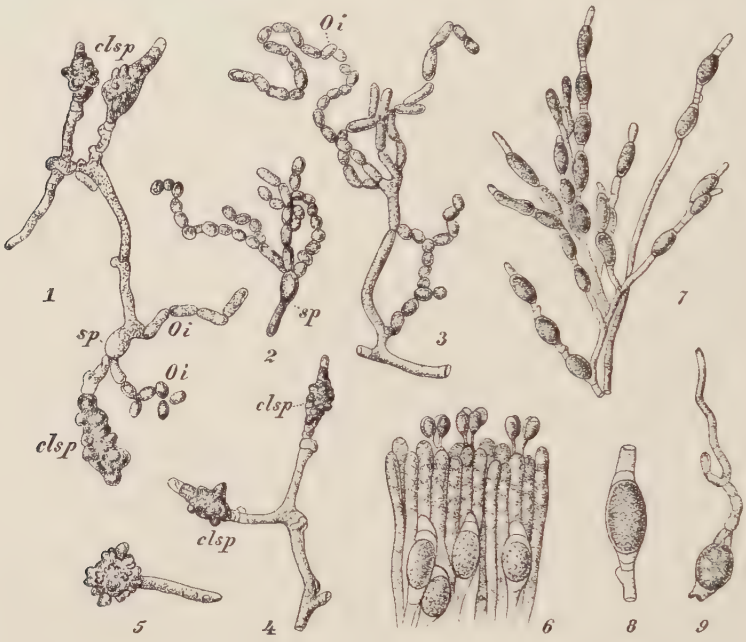


Fig. 189.—Nos. 1–5, *Nyctalis asterophora* (= *N. lycoperdoides* Bull.). No. 1, a small mycelium, grown from a basidiospore *sp*, with clamp-connections at the cross-walls, bearing chains of oidia *oi*, and chlamydospores *clsp*. No. 2, a small mycelium, grown from a basidiospore *sp*, the hyphae of which have become completely broken up into oidia. No. 3, a piece of mycelium breaking up into oidia *oi*. No. 4, a piece of mycelium with two chlamydospores *clsp*, and clamp-connections at the cross-walls. No. 5, a chlamydospore producing a germ-tube. Nos. 6–9, *Nyctalis parasitica*. No. 6, a transverse section through the hymenium, showing basidia bearing basidiospores and chlamydospores lying between them. No. 7, mycelium, grown from a basidiospore, and bearing chlamydospores. No. 8, a hypha bearing a ripe chlamydospore, more highly magnified. No. 9, a chlamydospore germinating. After Brefeld, from von Tavel's *Vergleichende Morphologie der Pilze*. Magnification : Nos. 1–6 and 9, 350 ; No. 7, 180 ; No. 8, 450.

(1) that the *chlamydospores* taken from a pileus germinate readily on living Russulae, (2) that the *basidiospores*, when produced, germinate readily in a Russula decoction, and (3) that the mycelium produced from a basidiospore breaks up into *oidia* which in their turn readily germinate, eventually producing typical stellate chlamydospores.

Since (1) smaller or less vigorous fruit-bodies either produce no gills at all or, if they produce gills, have an aborted hymenium, basidial production being limited to exceptionally large and vigorous fruit-bodies in which the chlamydospores are formed relatively late; since (2) all fruit-bodies, both large and small, normally produce chlamydospores in abundance; and since (3) the chlamydospores germinate readily on the living pilei of the host-plant; it seems fairly certain that, under natural conditions in the open, *Nyctalis asterophora* normally reproduces itself by means of its chlamydospores, in this respect doubtless resembling *Ptychogaster albus*. However, it also seems likely that the basidiospores, when formed and disseminated, may still serve for the direct infection of new host-plants, although, as yet, this supposition does not appear to have been put to the test of actual experiment. The part played by the oidia in the dissemination of the parasite, if any, is problematical.

It is not without interest to consider Brefeld's work on Nyctalis in the light of what we have recently been taught about sex in the Hymenomycetes by Mlle Bensaude,[1] Hans Kniep,[2] and certain other experimental cytologists.[3] Basing our remarks on Brefeld's observations, we can say that on germination a basidiospore of *Nyctalis asterophora*, like basidiospores in general, gives rise to a *primary* mycelium, *i.e.* a mycelium (1) having nuclei in the haploid or unpaired condition, (2) having simple cross-walls, and (3) producing oidia; and that, after a time, this primary mycelium gives rise to a *secondary* one, *i.e.* a mycelium (1) having nulcei in the diploid or paired condition, with conjugate nuclear division, (2) having the cross-walls provided with clamp-connections, and (3) not producing oidia. Since Brefeld's illustrations (Fig. 189, no. 1, p. 456) clearly indicate that a single basidiospore of *N. asterophora* produces a mycelium which, without any other mycelium, soon develops

[1] Mathilde Bensaude, *Recherches sur le cycle évolutif et la sexualité chez les Basidiomycètes*, Nemours, 1918, pp. 1–189.

[2] Hans Kniep, "Über morphologische und physiologische Geschlechtsdifferenzierung," *Verhandl. der Physikal.-med. Gesellschaft zu Würzburg*, 1919, pp. 8–16.

[3] The problem of sex in *Hymenomycetes* will be dealt with somewhat fully in vol. iv of these *Researches*. For British papers on the subject, *vide* Irene Mounce: (1) "Homothallism and the Production of Fruit-bodies by Monosporous Mycelia in the Genus Coprinus," *Trans. Brit. Myc. Soc.*, vol. vii, 1921, pp. 198–217; (2) "Homothallism and Heterothallism in the Genus Coprinus," *ibid.*, 1922, pp. 256–269.

clamp-connections, *i.e.* become secondary, there can be but little doubt that the fungus is *homothallic* and not heterothallic. Moreover, it is also clear that the stellate *chlamydospores are produced only in a secondary mycelium* and never in a primary one. It is therefore to be supposed that each chlamydospore contains at least one pair of nuclei, (+) and (−). If now under natural conditions, as seems probable, *N. asterophora* is propagated from generation to generation by means of chlamydospores, the mycelium throughout such successive generations doubtless remains continuously secondary in its character. However, whenever basidiospores are produced, the pair of nuclei, (+) and (−), cut off in each young basidium, fuse together, thus completing a sexual act, the fusion being followed by two successive nuclear divisions accompanied by nuclear reduction. Thus, as usual in Hymenomycetes, the basidiospores of *N. asterophora* come to be haploid in their nature and, on germination, give rise to haploid mycelia. We must therefore conclude that, in *N. asterophora*, it is only when basidiospores are produced that the sexual act is completed; whilst, when the fungus is propagated from generation to generation by chlamydospores alone, the completion of the sexual act (owing to the life-history not including basidiospore-production) is rendered impossible. A similar condition doubtless occurs in *Ptychogaster albus*.

In the Uredineae, as is now well known, the mycelium which gives rise to the uredospores, the uredospores themselves, and the mycelia which the uredospores produce all contain pairs of nuclei which divide conjugately, *i.e.* they are all in the secondary or diploid sexual phase, while it is only in the teleutospore that nuclear fusion takes place, thus completing the sexual act. When, therefore, a Rust fungus, *e.g. Puccinia Iridis*, *P. epiphylla*, or *P. Chrysanthemi*, omits its teleutosporic stage and propagates itself solely by uredospores, it is clear that its mycelium remains continuously secondary in character throughout successive generations, thus affording us an exact parallel with *Nyctalis asterophora* when propagated from generation to generation by chlamydospores alone.[1]

[1] Concerning Rust fungi which produce teleutospore-sori only under special conditions, my colleague, Dr. G. R. Bisby, has been good enough to supply me with the following information: "There are several rusts which seldom produce telia,

Nyctalis parasitica.—*Nyctalis parasitica* (Fig. 190), although resembling *N. asterophora* in being parasitic on certain other agarics, is quite distinct from that species. It is thus described by Rea :[1]

" Pileus 1·5–3 cm., *whitish fuscous*, then *becoming pale whitish*, somewhat fleshy, conico-campanulate, then convex, plane, obtuse or obsoletely umbonate, unequal, *pellicle persistent*, *pruinose*, *grey*. Stipe 2·5–6 cm. × 2–4 mm., *white*, straight or curved, equal, or slightly attenuated upwards, pubescent, bristling with hairs at the base. Gills *white*, *becoming fuscous*, adnate, thick, distant, with alternate shorter ones intermixed, at length contorted and anastomosing. Flesh *dark grey*. Spores white, elliptical, 5–7 × 3–4 μ. Smell like *Polyporus squamosus*. On dead *Russula adusta*, *Russula foetens*, *Russula chloroides*, and *Lactarius vellereus*. August–December. Common."

Among the hosts of *Nyctalis parasitica*, in addition to those recorded by Rea, are *Lactarius blennius* observed by Fries,[2] *Russula delica* observed by de Bary,[3] and *R. nigricans* observed by Brefeld.[4] Among the hosts which may be attacked by both *Nyctalis asterophora* and *N. parasitica* are : *Russula adusta*, *R. nigricans*, and *Lactarius vellereus*.

A photograph showing *Nyctalis parasitica* on *Lactarius vellereus* is reproduced in Fig. 190. For coloured illustrations the reader is referred to the works of Brefeld [5] and Cooke.[6]

In the description of the fruit-body of *Nyctalis parasitica* given

and a number (including many tropical forms) in which telia are not known. *Puccinia Iridis* (*North American Flora*, vol. vii, p. 379) is fairly common on *Iris versicolor* in the eastern part of North America, and upon this host is only found in the uredinial stage ; upon other species of Iris west of the Rocky Mountains telia may be found. *P. epiphylla* (*ibid.*, p. 327), in North America, produces telia and aecia only in alpine and boreal stations, although the uredinia occur almost throughout the continent. *P. Chrysanthemi* (*ibid.*, p. 508) is known over much of the world, but telia are known only in Japan. The formation of the telia has been shown by Raines (*American Journal of Botany*, 1922) to be partially at least a factor of the constitution of the fungus ; he was able to select cultures of *P. coronifera* with small or with large production of telia."

[1] Carleton Rea, *British Basidiomycetae*, Cambridge, 1922, p. 541.

[2] E. Fries, *Systema Mycologicum*, Gryphiswaldiae, 1821, vol. i, p. 136.

[3] A. de Bary, " Zur Kenntniss einiger Agaricinen," *Bot. Zeit.*, Bd. XVII, 1859, p. 393.

[4] O. Brefeld, *Untersuchungen über Pilze*, Heft VIII, 1884, p. 71.

[5] O. Brefeld, *ibid.*, Tab. VI, Fig. 1.

[6] M. C. Cooke, *Illustrations of British Fungi*, Plate 1113.

above Rea has omitted two points of fundamental importance for

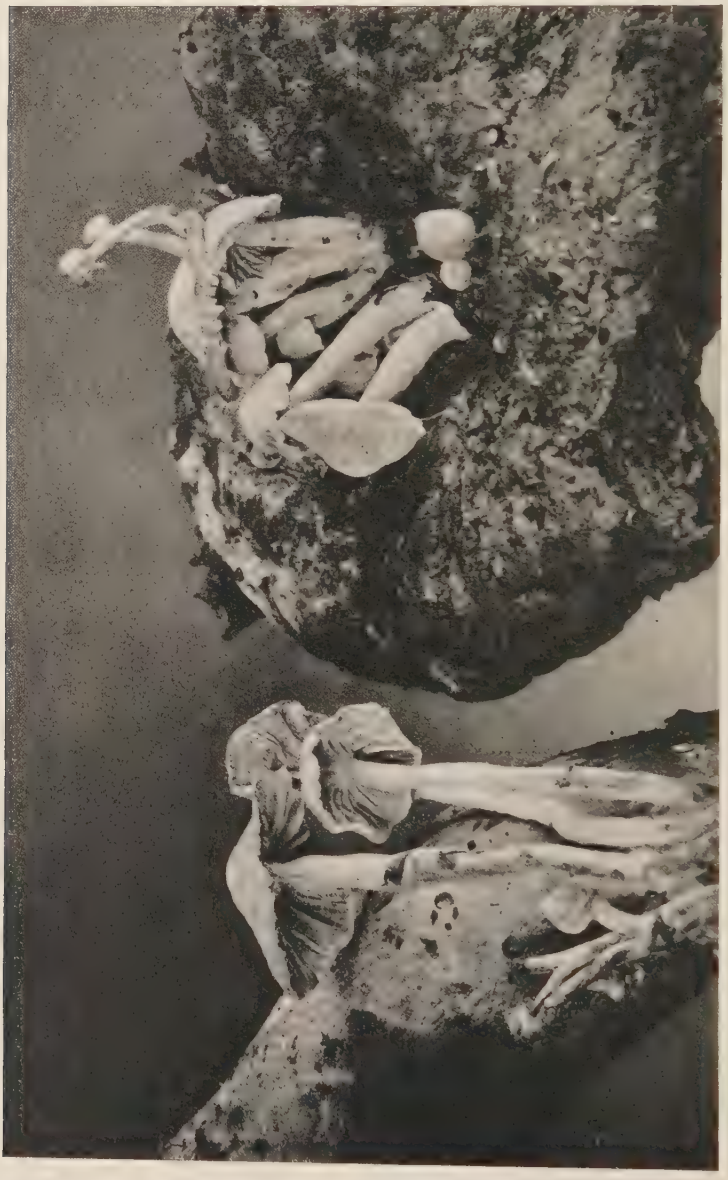


FIG. 190.—*Nyctalis parasitica* growing on *Lactarius vellereus*. The chlamydospores of the parasite are situated within the substance of the gills. Photographed in Yorkshire, England, by A. E. Peck. Natural size.

understanding the life-history of the fungus, namely, (1) the presence in the gills, but not in the pileus-flesh, of numerous chlamydospores

(Fig. 191, C, E), and (2) the fact that the fruit-bodies, so far as basidiospores are concerned, are usually quite sterile (Fig. 191, D, E, F).

The chlamydospores of *N. parasitica* are smooth elliptical bodies occurring at the ends of, and along the course of, the hyphae in which they are formed (Fig. 191, E, F, G, and Fig. 189, nos. 6-9, p. 456). In a transverse section through a mature gill they can readily be seen in the hyphae of the trama (Fig. 191, D), and Brefeld [1] found them invading even the hymenium (Fig. 189, no. 6, p. 456), thus resembling the chlamydospores of a fertile fruit-body of *Ptychogaster citrinus*.[2]

De Bary [3] observed that chlamydospore-formation begins in very young pilei which are scarcely 1 line broad and continues almost to the end of the fruit-body's existence (Fig. 191, A, B, C). New chlamydospores are formed: (1) immediately under the ends of the hyphae of the hymenium, which grow in length slowly and continuously and thus add to the thickness of the hymenium (Fig. 191, E, F), and (2) in hyphae which grow into the older gill-layers which already contain chlamydospores, branch there, and so thicken the gills and assist in flattening the pileus. In the end, the substance of each gill consists for the most part of chlamydospores which, since they are present in such large numbers, impart to the gills a dark-brown colour. The hyphae which bear the chlamydospores become very pale and largely disappear, thus setting the chlamydospores free. Hence ripe chlamydospores readily fall from the gills in the form of a brown powder.[4] This, doubtless, is carried away by the wind.

So far as basidiospores are concerned, the hymenium of most *N. parasitica* fruit-bodies is completely abortive, *i.e.* its elongated, outwardly directed, basidia-like cells produce neither sterigmata nor spores (Fig. 191, D, E, F).

De Bary [5] examined hundreds of pilei but could never find any basidiospores whatsoever, and my own attempts in this direction

[1] O. Brefeld, *loc. cit.*, p. 94.

[2] *Cf.* Fig. 69, no. 2, p. 154, in F. von Tavel's *Vergleichende Morphologie der Pilze*, Jena, 1892. This figure, taken from Brefeld's *Untersuchungen*, will be reproduced in vol. iv.

[3] A. de Bary, *loc. cit.*, p. 395. [4] *Ibid.* [5] *Ibid.*

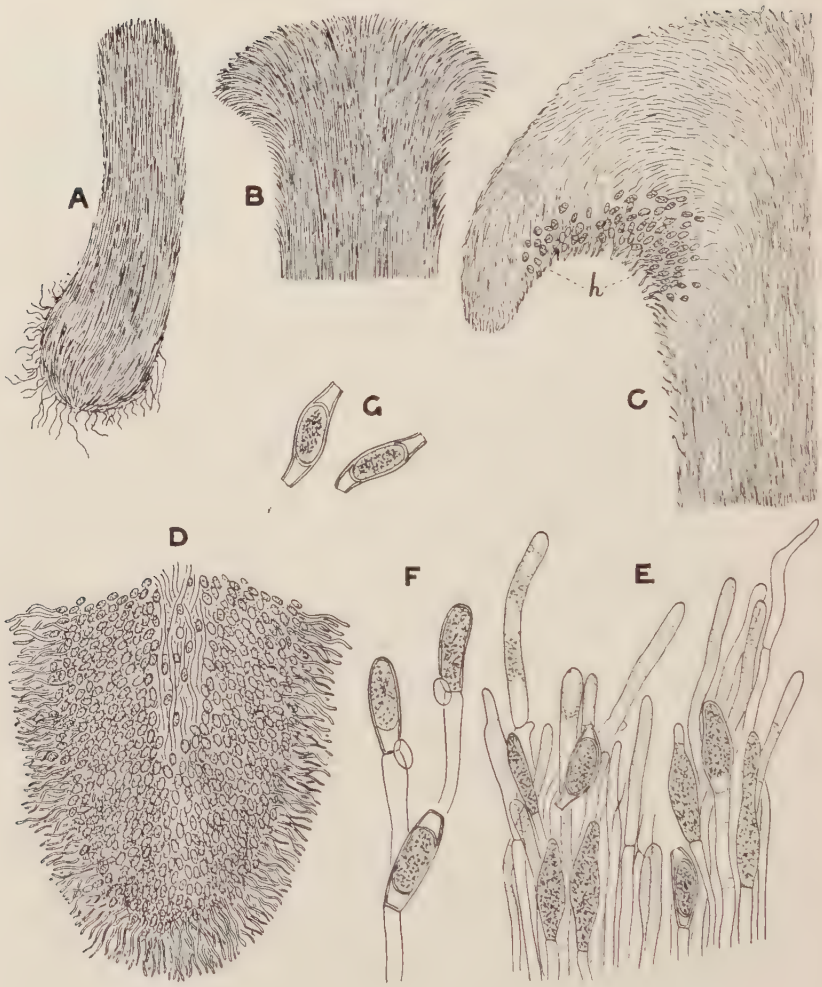


FIG. 191.—*Nyctalis parasitica*. A, young fruit-body before the formation of the pileus-rudiment. Magnification, 45. B, longitudinal section of the top of a slightly older fruit-body with the pileus developing. Magnification, 90. C, longitudinal section through a somewhat older pileus; already the hymenium, *h*, contains numerous chlamydospores. Magnification, 90. D, transverse section through a gill of an older fruit-body, showing numerous chlamydospores. Magnification, about 100. E, a thin section through the outer part of a gill, showing loose hyphae forming chlamydospores. F, single hyphae isolated from the hymenium, showing stages in chlamydospore-formation. G, two ripe chlamydospores which have escaped from a gill. E, F, and G, magnification, 390. The drawings copied by Dr. Nellie Carter from de Bary's paper in the *Botanische Zeitung* (Bd. XVII, 1859) and arranged by the author.

have also completely failed. Brefeld[1] states that fertile fruit-bodies of *N. parasitica* are much more rare than those of *N. asterophora*, but that in one very vigorous fruit-body he did actually observe basidia bearing sterigmata and spores (Fig. 189, no. 6, p. 456). This instance of fertility, however, must be regarded as very exceptional.

Brefeld[2] succeeded in germinating the basidiospores and chlamydospores of *Nyctalis parasitica* with results similar to those obtained for *N. asterophora*. A basidiospore, on germination, gave rise to a mycelium bearing oidia, and the oidia germinated and produced a mycelium bearing more oidia and finally chlamydospores in chains (Fig. 189, no. 7). A chlamydospore, on germination, gave rise to a mycelium devoid of oidia (Fig. 189, no. 9). From the point of view of sex, the life-history of *N. parasitica* appears to resemble exactly that of *N. asterophora*.[3]

Since the fruit-bodies of *Nyctalis parasitica* always bear chlamydospores in their gills and but very rarely produce basidiospores on their hymenium, one must infer that, normally, under natural conditions in the open, the fungus is disseminated and reproduced by means of its chlamydospores.

The only other agaric with which *Nyctalis parasitica* can possibly be confused in the field is *N. asterophora*. It may therefore be of interest to contrast the two species. As compared with *N. asterophora*, *N. parasitica* differs in the following particulars: (1) the stipe is often considerably longer; (2) the pileus is more expanded, less fleshy for its size, and often broader; (3) the gills are often fairly well developed, and never absent; (4) the chlamydospores are formed, not in the pileus-flesh, but in the gills, so that the upper stratum of the pileus does not break up into a fawn-coloured powdery mass, but remains firm and covered by a persistent pellicle; and (5) the chlamydospores are always smooth and elliptical, and never stellate.[4]

[1] O. Brefeld, *loc. cit.*, pp. 93–94. [2] *Ibid.*, p. 94. [3] *Cf.* pp. 457–458.

[4] Rea (*Brit. Bas.*, p. 541) says that *N. parasitica* has a smell like *Polyporus squamosus*, but he does not mention the scent of *N. asterophora*. On the other hand, de Bary (*Bot. Zeit.*, Bd. XVII, p. 393, *cf.* also p. 385) expressly states that *N. parasitica* has the same smell of meal as *N. asterophora*. The two species cannot therefore be separated by their smell.

Stropharia epimyces.—*Stropharia epimyces* is a North American parasite which confines its attack to the fruit-bodies of two of the largest Coprini, namely, *C. comatus* and *C. atramentarius*. For illustrations of *S. epimyces* on *Coprinus comatus* and for a discussion of its parasitism the reader is referred to Chapter VII (Figs. 65 and 66, pp. 165 and 167). Here I shall simply add a few remarks of a comparative nature.

Stropharia epimyces appears to be the only agaric parasitic on other agarics which always completely masters its host. The Coprinus fruit-bodies attacked lose their characteristic shape, become dwarfed, are rendered more or less sterile, and never expand, while their nutrient substances are exhausted by the parasite to form fruit-bodies of its own. As a rule the other agarics parasitic on agarics—*Boletus parasiticus*, *Leptonia parasitica*, *Claudopus subdepluens*, *Volvaria Loveiana*, *Nyctalis asterophora*, and *N. parasitica* —deform their hosts either but slightly or not at all and doubtless allow them to produce at least some spores from the hymenium, whether this be situated within their gleba (Scleroderma), on the inner surface of their hymenial tubes (*Polyporus perennis*), or upon the exterior of their gills (*Cantharellus cibarius*, *Clitocybe nebularis*, Russulae, and *Lactarius vellereus*). In completely mastering its host, *Stropharia epimyces* exhibits a high specialisation which is analogous with that of *Hypomyces lactifluorum*[1] on *Lactarius piperatus* and of *Ustilago tritici* on wheat.

It may be asked: why does *Stropharia epimyces* attack only certain Coprini; *Volvaria Loveiana* only certain species of *Clitocybe*; and the parasitic species of Nyctalis only certain Russulae, Lactarii, etc.? The answer to this question, if we knew it, would probably be in chemical terms. Doubtless, where a parasite is specialised to attack a single host or a few related hosts, the chemical juices of the host or hosts must afford a very perfect nutrient medium to the hyphae of the parasite and be without any toxic or other detrimental effects upon them—a combination which may be rare. Also for successful parasitism the juices of the host must either act upon the hyphae of the parasite in a positively chemotropic manner, or at least be of such a nature as to permit the hyphae of the parasite to

[1] *Cf.* these *Researches*, vol. ii, 1922, pp. 58–69.

grow in contact with the hyphae of the host without any chemotropic repulsion. For further advances in our knowledge of the parasitism of agarics which live on other agarics we must look, in the main, to the biochemist.

Collybia dryophila and its Pseudoparasite Tremella mycetophila.—*Collybia dryophila* is a well-known agaric commonly found in woods both in Europe and North America. In its general appearance it is reminiscent of *Collybia butyracea*, *C. velutipes*, and some other species of Collybia; but it has the property of reviving in wet weather after being dried[1] and has therefore been placed by Rea[2] and some other systematists in the genus Marasmius. Its spores are white and, on the average, measure $5{\cdot}4 \times 3{\cdot}2\ \mu$.[3] A single spore begins its development on the end of a sterigma, grows to full size, matures, and is discharged all within a space of time which somewhat exceeds one hour.[4] When a dried up fruit-body is wetted by a shower of rain, it very quickly absorbs water through the upper surface of its pileus, its basidia then resume their development and, within an hour or two, the pileus begins to liberate spores once more.

The gills of *Collybia dryophila* are crowded together. When in an expanded lamellated fruit-body the gills are distinctly crowded, the spores are usually of small size; and, generally, in the Agaricineae, there seems to be a correlation between the crowding of the gills and the size of the spores of such a nature that, on the whole, *the more crowded the gills, the smaller the spores*.[5] Thus, to take extreme examples, in *Collybia dryophila*, *C. maculata*, *Clitocybe nebularis*, and *Panus stypticus*, all of which have crowded gills, the size of the spores is 5–6 × 3–4 μ, 5–6 μ, 7–8 × 3–4 μ, and 4–5 × $2{\cdot}25\ \mu$, respectively; while in *Collybia radicata*, *C. platyphylla*, *Gomphidius viscidus*, and *Amanitopsis vaginata*, all of which have relatively distant gills, the size of the spores is 14–15 × 8–9 μ,

[1] These *Researches*, vol. i, 1909, pp. 107, 108, 111.

[2] Carleton Rea, *British Basidiomycetae*, Cambridge, 1922, p. 524.

[3] These *Researches*, vol. i, p. 162.

[4] These *Researches*, vol. ii, 1922, p. 49.

[5] Small spores are correlated with small basidia, and large spores with large basidia (*cf.* vol. ii, p. 412), so that one might also say: *the more crowded the gills, the smaller the basidia.*

8–10 × 6–8 μ, 18–22 × 6–7 μ, and 10–12 μ, respectively.[1] We thus see that *Collybia dryophila*, in having crowded gills associated with small spores, follows a rule which, although perhaps not without its exceptions, very generally holds among the Agaricineae.

In most fruit-bodies of *Collybia dryophila* the surface of the pileus and the exterior of the stipe are quite smooth. Occasionally, however, in the United States of America (New York, New Hampshire, Vermont, Minnesota)[2] and in Canada (Ontario, Manitoba),[3] as well as very rarely in Europe (France),[4] fruit-bodies are found in which both the pileus and the stipe bear curious excrescences which at once suggest the presence of a parasite (Figs. 192, 193, 194, A and B). Peck[5] met with some of these abnormal fruit-bodies in the State of New York and, in 1879, he described the excrescences as *Tremella mycetophila*.

Fig. 192.—*Collybia dryophila*, with stripes bearing Tremella-like outgrowths formerly believed to be due to a parasite, *Tremella mycetophila*. Collected at Ottawa, Canada, by W. S. Odell. Photographed by the Photographic Division of the Canadian Geological Survey. Natural size.

The excrescences are suborbicular, depressed, much contorted or gyrose-plicate, fleshy, pruinose, yellowish or pallid bodies which

[1] The dimensions of the spores here given are taken from Rea's *British Basidiomycetae*, Cambridge, 1922.

[2] *Vide infra*, the papers of E. A. Burt and Miss Hone.

[3] I myself have found the excrescences on *Collybia dryophila* in woods near Winnipeg, Manitoba, and Mr. W. S. Odell in woods near Ottawa, Ontario.

[4] *Vide infra*, the paper of E. Boudier.

[5] C. H. Peck, *Report of the New York Museum*, vol. xxviii, 1879, p. 53, Plate I, Fig. IV.

vary in breadth from about 0·3 to 1 inch and upwards. Their outer surface is covered with a hymenium containing simple cylindrical 4-spored basidia.

In 1900 Atkinson,[1] accepting Peck's view of the nature of the excrescences, described and illustrated them from specimens which he found near Ithaca. "This plant," says he, "is interesting from the fact that it is parasitic on a mushroom, *Collybia dryophila*."

In 1901 Burt [2] published a paper on the structure and nature of *Tremella mycetophila* in which he pointed out that the excrescences could not be considered as belonging to a Tremella because: (1) their substance is not truly gelatinous like that of the fruit-bodies of the Tremellaceae but is fleshy (Fig. 194, D), as may well be seen when the excrescences are allowed to dry; and (2) their basidia are not longitudinally and cruciately divided by walls, but are simple and undivided like those of the Hymenomycetes in general (Fig. 194, C). Burt therefore came to the conclusion that the fungus "should not be included in the Tremellaceae but in the Thelephoraceae." He transferred the plant to the genus Exobasidium and called it *Exobasidium mycetophilum* (Peck) Burt.

Burt [3] found that the excrescences produce not only basidia on their exterior, but also conidia within their context (Fig. 194, D, E, F). The dimensions of the basidiospores and of the conidia he gives as 5–7 × 1·5–2·5 μ and 2 × 1·5 μ respectively. The conidia were present in the specimens which he collected during eight seasons in two widely-separated localities, one in New York State and the other in Vermont.

In 1909, in the State of Minnesota, Miss Hone [4] found the supposed Exobasidium, again on the cap and stipe of *Collybia dryophila* (Fig. 193). Some of the gyrose excrescences were of extraordinary size. She says "The fungus masses have very much the appearance of a Tremella and were so large that they extended in folds over the

[1] G. F. Atkinson, "Mushrooms, Edible and Poisonous, etc.," Ithaca, 1900, pp. 204–205.

[2] E. A. Burt, "Structure and Nature of *Tremella mycetophila* Peck," *Bull. Torrey Bot. Club*, vol. xxviii, 1901, pp. 285–287.

[3] *Ibid.*, p. 286.

[4] D. S. Hone, "Two Basidiomycetes new to Minnesota: *Exobasidium mycetophilum* and *Cantharellus retirugus*," *Minnesota Botanical Studies*, 1909, pp. 61–63.

surrounding chips and earth in which the Collybia was growing. One mass was four inches in diameter. The Collybia was growing abundantly among the decaying débris of a large water-soaked log and was covered over with the white convoluted masses of the Exobasidium." Miss Hone [1] remarks that her Minnesota specimens differ from those described by Burt in (1) the much larger size of

FIG. 193.—*Collybia dryophila*, with stipes and pilei bearing Tremella-like outgrowths formerly believed to be due to a parasite, *Tremella mycetophila*. Collected in Crow Wing County, Minnesota, U.S.A., and photographed by Miss D. S. Hone. Natural size.

the convoluted masses, some of which were 4 inches in diameter, and in (2) the more nearly spherical shape and much smaller size of the basidiospores. The basidiospores observed by Miss Hone were only 2-3 μ in diameter, whereas those of Burt, as we have seen, were 5–7 $\times$ 1·5–2·5 μ.

In 1915, in his account of Exobasidium, Burt [2] excluded *E. mycetophilum* from the genus and gave to the supposed parasite a new interpretation. Instead of regarding the excrescences as belonging

[1] D. S. Hone, *loc. cit.*, p. 63.

[2] E. A. Burt, "The Thelephoraceae of North America. IV. Exobasidium," *Ann. Missouri Bot. Gard.*, vol. ii, 1915, p. 656.

to a parasitic fungus, he expressed the view that they were nothing more than abnormal outgrowths of *Collybia dryophila* itself. "This curious structure on *Collybia dryophila*," says he, "I no longer regard as parasitic but, rather, as a teratological production of *C. dryophila*, induced by protracted wet weather during development of the fructification."

In 1917 Boudier[1] described *Exobasidium mycetophilum* from specimens found near Nancy in France, and his illustration shows a large, yellowish, almost globular, cerebriform excrescence covering the top of the pileus of a *Collybia dryophila* and a similar but much smaller excrescence protruding from the stipe. He states that the conidia produced within the flesh are "numerous, minute, rounded bodies, 1–2 μ in diameter" and that the basidia are "tetrasporous, evidently connected with the hyphae of the Collybia, and bear ovate spores similar to those of the Collybia but often somewhat larger."

Boudier's interpretation of the excrescences is similar to that of Burt; for he holds that they are not the fruit-bodies of a parasitic fungus, but rather "une modification pathologique due à une production conidifère du Collybia."

Also in 1917 C. G. Lloyd[2] published a note on the excrescences of *Collybia dryophila* accompanied by a photographic illustration. After stating Burt's view that Peck's *Tremella mycetophila* is nothing more than a teratological outgrowth, he adds: "we do not claim to know anything about what it really is. . . . If Burt's theory is correct it is an exceptional case. We have sometimes noted evident teratological development of Agarics forming abortive pilei on top of the normal pileus, like the celebrated *Poria agaricicola* that a German savant discovered on an Amanita. But we think that there is no other case where an Agaric habitually develops an entirely different and constant fruiting form in addition to its normal gills."

Some years ago, I myself found the supposed *Exobasidium mycetophilum* in a wood near Winnipeg; but, thinking that there would be no great difficulty in procuring it again, I examined it only superficially and kept no record of my observations.

[1] E. Boudier, "Dernières étincelles mycologiques," *Bull. Soc. Myc. France*, T. XXXIII, 1917, pp. 13–14, Plate II, Fig. II.

[2] C. G. Lloyd, *Mycological Writings*, Cincinnati, vol. v, No. 47, 1917, p. 662.

Unfortunately I have never met with it since. In English woods, especially those of Sutton Park near Birmingham, in the course of twenty years, I must have seen some thousands of *C. dryophila*

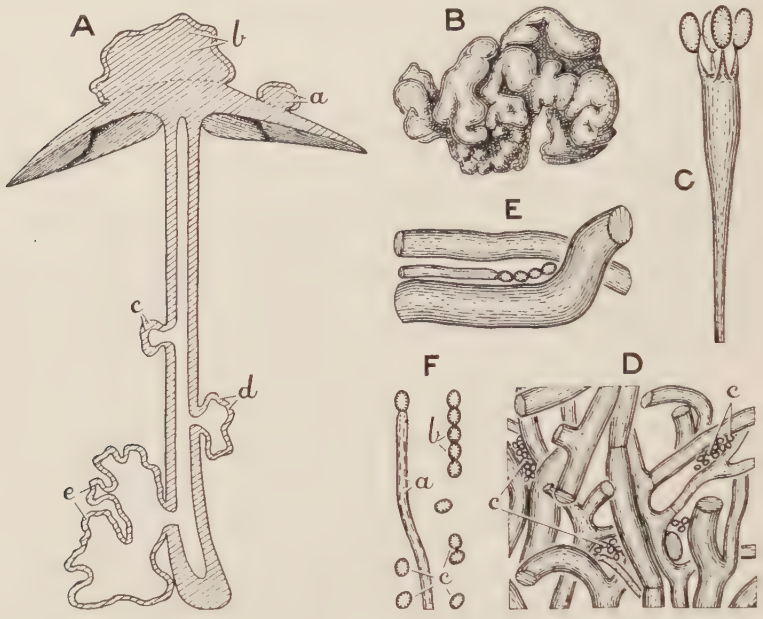


FIG. 194.—Abnormal tremelloid outgrowths of the pileus and stipe of *Collybia dryophila*, formerly known as *Tremella mycetophila* Pk. A, a diagrammatic vertical section through a fruit-body of *C. dryophila* showing two solid outgrowths, *a* and *b*, on the top of the pileus and three hollow outgrowths, *c*, *d*, *e*, on the stipe. B, a large outgrowth, removed from a stipe, showing the cerebriform hymenial surface. C, a basidium from the hymenium covering an outgrowth such as B. D, a section through the deeper tissue of an outgrowth showing hyphae with non-gelatinous walls and groups of conidia, *c c*. E, four conidia at the end of a slender hypha in the deeper tissue of an outgrowth. F, various conidia from the interior of an outgrowth: *a*, one borne at the end of a slender hypha; *b*, five in a chain; *c*, several isolated from one another. A and B, natural size; C, E, and F, magnification, 760; D, magnification, 333. A, original; B–F, after E. A. Burt, redrawn and arranged by the author.

fruit-bodies, but never once have I observed a single specimen which bore excrescences like those which Peck called *Tremella mycetophila*. My own experience with *Collybia dryophila* in England is similar to that of other mycologists; for, so far as I know, the supposed parasite has never yet been recorded for that country.

It seems to me that Burt and Boudier have correctly interpreted

the excrescences. In other words, I believe that the supposed parasitic fungus *Exobasidium mycetophilum* does not exist but that, under certain conditions, *Collybia dryophila* develops on its pileus and stipe teratological structures or intumescences which look deceptively like a Tremella. It is on this account that, in the heading of this Section, I have called *E. mycetophilum* a *pseudo-parasite.*

Burt, as we have seen, has expressed the view that the excrescences are " induced by protracted wet weather during development of the fructification " ; but, with Lloyd,[1] I am disinclined to accept this explanation. As we know, England has a very damp climate and often experiences protracted wet weather during the development of *Collybia dryophila* fruit-bodies, while the climate in Manitoba is relatively very dry. Yet the excrescences occur in Manitoba but not in England, which is the opposite of what we should expect on Burt's theory. It has yet to be determined whether the excrescences are inherited from one generation to another or come into existence only as a result of some special, occasionally applied, external stimulus. It may be that there are races of *Collybia dryophila* which are especially prone to become malformed. However, although at present we do not know what is the true cause of the origin of the excrescences, their existence reveals to us an unsuspected plasticity in the fruit-body of an agaric which was previously believed to possess a form that could only vary within very restricted limits.

A Morchelloid Modification of Collybia velutipes.—In concluding this Chapter, attention may be called to the fact that, in addition to *Collybia dryophila*, there is another Collybia which sometimes has abnormal fruit-bodies, namely, *C. velutipes.*

A teratological form of *Collybia velutipes* was found in France and described by Legué[2] in 1909. In this the pileus, although provided with gills, has an alveolate upper surface, reminding one of a little *Morchella esculenta* (Fig. 195). In a normal pileus (Fig. 202, p. 490 ; also Vol. II, Fig. 15, p. 47) the hymenium, of course, is

[1] C. G. Lloyd, *loc. cit.*, p. 662.

[2] L. Legué, " Note sur une forme anomale de *Collybia velutipes* Curt.," *Bull. Soc. Myc. France*, T. XXV, 1909, pp. 120–122.

confined to the gills; but, in our morchelloid pileus, it not only covers the gills, but also lines the outer surfaces of all the alveoli on the top of the pileus.

The cause of the morchelloid development of the pileus in Legué's *Collybia velutipes* fruit-bodies is as much a mystery as the cause of the occasional tremelloid development of the pileus and stipe in fruit-bodies of *C. dryophila*; but, here again, we are pro-

FIG. 195.—*Collybia velutipes*, a morchelloid form observed in France. The alveolate upper surface of the pileus, like the gills, is covered with a fertile hymenium. Photographed by the late L. Legué. Natural size.

vided with evidence of remarkable plasticity in a species which normally varies within very narrow limits.[1]

Another teratological form of *Collybia velutipes* was found in the State of New York by Stewart[2] in 1911. "The malformation," says Stewart, "was one affecting the gills. Instead of being straight and even in the edge like a knife-blade, as is the case with normal gills, they were irregular and double-edged, with numerous lateral projections and a strong tendency to anastomose." Three of the

[1] *Collybia velutipes* is not the only agaric which occasionally develops in a morchelloid manner. Thus morchelloid forms of certain Cortinarii have been described by Boudier (*Bull. Soc. Myc. Fr.*, T. VI, p. 169 and Plate XVIII) and by Dumée and Lutz (*Ibid.*, T. XVIII, p. 131).

[2] F. C. Stewart, *The Velvet-stemmed Collybia—a Wild Winter Mushroom*, Bull. no. 448, New York Agri. Exp. Station, 1918, p. 83.

malformed fruit-bodies were found among a large number of normal ones growing in a cluster at the base of a dead willow tree ; and a week later, in the same place, ten additional plants were observed showing the same malformation to a greater or less extent.

Still another teratological form of *Collybia velutipes* was observed by Stewart,[1] namely, " a robust young sporophore with four small sporophores forming on the gills. The pilei of the secondary sporophores were perfect in form, but almost sessile. Three of them were 2 mm. in diameter, while the fourth was smaller. With the aid of a hand-lens the gills were readily distinguishable. No evidence of mutilation of the gills of the primary sporophore was detected." I [2] have observed secondary sporophores on the gills of *Lentinus lepideus* in fruit-bodies growing on wood in the laboratory ; and Brefeld [3] has described and illustrated a similar phenomenon in *Coprinus stercorarius*. However, Brefeld ascribes his secondary sporophores to the effect of wounds made in the mother-sporophore when it was very young.

While the teratological forms of *Collybia velutipes* observed by Stewart concern the gills only and are not so striking to the eye as the morchelloid modification described by Legué, they nevertheless afford additional evidence of the plasticity of the fruit-bodies affected and of apparently autonomous changes in the usual mode of morphogenesis.

[1] F. C. Stewart, *loc. cit.*, p. 85.

[2] A. H. R. Buller, " The Reactions of the Fruit-bodies of *Lentinus lepideus* to External Stimuli," *Annals of Botany*, vol. xix, 1905, p. 434. Also *cf.* these *Researches*, vol. i, 1099, p. 48, Fig. 16, I.

[3] O. Brefeld, *Untersuchungen über Pilze*, Leipzig, Basidiomyceten I, 1877, pp. 72–75.

CHAPTER XIV

THE NOCTURNAL SPORE-DISCHARGE OF PLEUROTUS OSTREATUS AND COLLYBIA VELUTIPES AS OBSERVED WITH AN ELECTRIC HAND-LAMP

The Electric Hand-lamp and its Employment in the Field—*Pleurotus ostreatus*—The Effects of High and Low Temperatures—November and December Field-observations—*Collybia velutipes* and its Activity in Mid-winter

The Electric Hand-lamp and its Employment in the Field.—In Volume I. of these *Researches* I described a beam-of-light method for rendering visible the spores falling from a hymenomycetous fruit-body in the laboratory.[1] It was shown that, if one suspends such a fruit-body in a closed beaker and directs a strong beam of light through the air below the pileus, then, with the naked eye, one can see the individual spores streaming away from the gills. The existence of the spore-stream, thus revealed, affords striking evidence of the intense activity with which a fruit-body carries out its one great function of producing and liberating spores; and, when once such a spore-stream has been seen, it is not likely to be forgotten. Recently, for the purpose of observing the discharge of spores from fruit-bodies growing in the open under natural conditions, I have employed a modification of the beam-of-light method; and the essential conditions for using this modified method with success will now be set forth:

(1) One must first find some fruit-bodies shedding spores. Particularly convenient for observation are lignicolous fungi, such as *Pleurotus ostreatus* and *Collybia velutipes*, when found growing on the side of a tree-trunk a few feet above the ground and therefore well within reach.

[1] These *Researches*, vol. i, 1909, pp. 94–101.

(2) One needs a source of light. I have obtained good results with an electric hand-lamp, such as is made use of in garages, etc. This hand-lamp (Fig. 196) is merely an upright rectangular wooden box ($2{\cdot}5 \times 3{\cdot}5 \times 7{\cdot}5$ inches), containing a storage battery and a filament, having a leather handle above, and emitting its light through a laterally situated plano-convex lens.

Fig. 196.—The electric hand-lamp used for nocturnal observations on the spore-discharge of Agaricineae. Reduced to about $\frac{2}{7}$. Diameter of lens, 2·25 inches.

(3) One also requires a dark background against which one may see the illuminated spores. To obtain this, one works by night instead of by day. At night, especially during the autumn and winter months, a dark or even deep black background of shadows may be readily procured.

(4) The temperature must not be below freezing-point; for, if it is, spore-discharge in all probability will have ceased, at least temporarily. As a rule, the warmer the air, the more rapidly does a fruit-body liberate its spores. Warm weather is therefore to be preferred to cold.

(5) Lastly, the wind must not be too strong, for a strong wind disperses the spores so rapidly that one may find it difficult or even impossible to perceive them. In windy weather one should screen the fruit-bodies from the wind with an overcoat. The best results are obtained on still nights.

Granted that the conditions for making observations are favourable, let us go out together on a little nocturnal expedition. We are in England, the month November. Of late it has been mild and even now the thermometer registers 45° F. The night is dark

and very still and, as we leave the house, not a sound do we hear but the momentary cry of a Brown Owl and the village clock in the distance striking the hour of midnight. Carrying our hand-lamp, we make our way down the garden path and along a winding mossy way to a Poplar which, when decapitated and dismembered a few years ago, became a prey to wood-destroying fungi. Here on the side of the trunk, three feet from the ground, cluster four large fruit-bodies of the Oyster Fungus. Let us turn our attention to the largest which projects freely beyond its fellows. I take the lamp and, feeling like Aladdin when about to summon the genie to grant a wish, I revolve the lens and lo! the beam of light appears. Now I hold the lamp so that its beam is directed just beyond the edge of the pileus. We look across the beam towards one of the night's black shadows. At first, perhaps, we see nothing unusual; but, as I move the lamp around the edge of the pileus, the beam comes to a place where, suddenly, we both perceive the spores in immense numbers streaming from the gills and pouring through the light (Fig. 200, D, p. 485). How silently and continuously do these myriads of microscopic particles hurry by! What clouds of witness to the fruit-body's activity! But all at once the spore-stream has disappeared. Evidently, the slight and intermittent breeze was sweeping the spores in one direction, and now the breeze has changed its course. So we move the lamp around the edge of the pileus until the spore-stream has come into the beam of light once more. Having accustomed ourselves to the appearance of the spore-cloud, we remove the lamp to the lee side of the fruit-body and, from a distance of two feet, direct the beam towards the rim of the pileus. In a moment or two, by trial, we find the exact place to which the spores are streaming, and now we are able to perceive the spore-clouds floating towards us and coming all the way across the gap which separates our lamp from the fungus. For some minutes longer we continue our observations, and then turn back to the house. Before retiring to bed, we sit by the fire discussing what our expedition has revealed: we agree that our interest in the Oyster Fungus has been deepened and are both fain to admit that never before had we realised how actively a hymenomycetous fruit-body produces and liberates its spores during the hours of darkness.

In the morning, one of us makes a strange confession : " I dreamed I was in a gloomy forest and stood watching dense volumes of spore-smoke being given out by the metabolic fires of a huge Toadstool ; but, just as the fire brigade was coming, I awoke."

At noon we again seek our Oyster Fungus. The temperature is even higher than last night, the air very still, and the light fairly good. Surely as many spores must be coming away from the gills per minute as were coming during our nocturnal visit. Yet we see none of them. The reason for our failure is that, although the spores are illuminated by the light of day, we lack the deep blackness of the night for our background.

Pleurotus ostreatus.—*Pleurotus ostreatus*, the Oyster Fungus, is lignicolous and forms its fruit-bodies in imbricating clusters on dead or dying trunks and branches of trees. In the United States it has been observed on Oak, Elm, Maple (Figs. 197 and 198), and Basswood (Tilia).[1] In England I have often seen it on Beech (Vol. I, Figs. 2 and 3, pp. 22 and 23), Poplar, and Holly, while, according to Berkeley,[2] it is especially common on Laburnum. In England it is distinctly a plant of the late autumn, for it is often at its best in November and, if the weather is mild, may be seen flourishing even in December. Learn,[3] writing from Ithaca in the State of New York, remarks that *P. ostreatus* is found from August to early December. In England, the pileus of the variety with which I am best acquainted is beautifully blue[4] during the first few days of its development ; but, as the pileus increases in size and becomes older, this blue colour gradually fades away and is replaced by yellowish-brown. The diameter of well-developed pilei varies from 8 to 20 cm. (3–8 inches). At first the margin of the pileus is incurved (Fig. 199, p. 483). The pileus extends itself by long-continued peripheral growth, so that the oldest parts of the gills are by the stipe and the youngest at the pileus-margin. If the weather is favourable, a single fruit-body may persist in good condition and shed spores

[1] C. D. Learn, " Studies on *Pleurotus ostreatus* and *Pleurotus ulmarius*," *Annales Mycologici*, vol. x, 1912, p. 542, also Plate XVI.

[2] M. J. Berkeley, *Outlines of British Fungology*, London, 1860, p. 136.

[3] C. D. Learn, *loc. cit.*

[4] This blue has been compared to that seen on the neck of a dove (Columba).

FIG. 197.—*Pleurotus ostreatus.* Groups of fruit-bodies growing on the trunk of a Silver Maple (*Acer saccharinum*). Diameter of the trunk about 2 feet 6 inches. Width of the upper central fruit-body cluster, about 8 inches. The coin on one of the medium right-hand fruit-bodies is a silver dollar. Photographed at Ithaca, New York State, on October 25, 1911, by C. D. Learn. About $\frac{1}{11}$ natural size.

continuously for a month. Berkeley [1] thus describes the fungus: "Pileus soft, fleshy, subdimidiate, conchate, ascending, turning pale; stem short or obsolete, firm, elastic, strigose at the base; gills decurrent, rather distant, anastomosing behind, dirty-white." And he adds: "On trees, especially laburnum. Late in the autumn and winter. Pileus cinereous." Atkinson [2] states that the pileus is "white, light grey, buff or dark grey, often becoming yellowish on drying." The name *Oyster Fungus* owes its origin to the fact that the form of the fruit-body sometimes suggests an Oyster shell.

In the summer of 1923, at Sutton Park, Warwickshire, I found some fully expanded fruit-bodies of *Pleurotus ostreatus* attached to the trunk of a Holly Bush (*Ilex Aquifolium*). As there had been no rain for about a month, the pilei were very hard and dry and the gills stiff and somewhat curled. Doubtless, during their development, the pilei had shed large numbers of spores. I took the fruit-bodies home, kept them dry for about a month, and then allowed them to absorb water through their upper surfaces. They soon revived and, in the course of the next few days, gave rise to copious spore-deposits. Evidently under natural conditions in the open, the fruit-bodies of *Pleurotus ostreatus*, like those of species of Lenzites, Polystictus, etc., may have their spore-discharge period temporarily interrupted by drought.

The observations about to be recorded were made by the author in November and December, 1922, near Birmingham, England, during a period of leave-of-absence from the University of Manitoba.

Among the late-autumn fungi in my father's garden at King's Heath the Oyster Fungus was conspicuous, for clusters of its fruit-bodies appeared on several Poplars (*Populus serotina*) from which a few years before all the main branches had been lopped off (Fig. 199, p. 483). The severe trimming to which the trees had been subjected had doubtless provided the conditions for the entry of the mycelium into the wood. All the trees appeared to be dying and some were already dead. Two of the latter had been cut down and removed to a wood-shed which was open on one side.

[1] M. J. Berkeley, *loc. cit.*, pp. 135–136.

[2] G. F. Atkinson, *Mushrooms, Edible and Poisonous, etc.*, Ithaca, U.S.A., 1900, p. 104.

The Effects of High and Low Temperatures.—On November 19, in the wood-shed, I found some large fruit-bodies of *Pleurotus ostreatus* projecting from the sides of one of the Poplar logs. The air was still and the diffuse daylight available fairly strong. On watching intently one of the largest fruit-bodies against a black background, I distinctly saw with the naked eye tiny white spore-clouds coming slowly away from the gills. On November 22 I repeated this observation. For November 19 and 22 the maximum temperatures were 46° F. and 44° F. respectively, and the minimum temperatures both 42° F.[1] At noon on November 25 and some other days when the temperature was decidedly lower, spore-discharge from the pileus was invisible. The evidence seemed to indicate that only on relatively warm days was the emission of the spores sufficiently rapid to enable one to perceive the spore-clouds. The only other species of Agaricineae from which I have as yet seen spores escaping from the gills under natural conditions is *Armillaria mellea*.[2]

During the night of November 24 there was a slight frost, and November 25 was a cool day with maximum and minimum temperatures of 40° F. and 28° F. respectively. At noon on November 24 I was unable to perceive spores being given off from the fruit-bodies on the log. At 1.30 P.M. I gathered one of the fruit-bodies and set it in a room where the temperature was about 70° F. At 9 P.M., with the help of a beam of light given out by the electric hand-lamp described in the preceding Section, I was easily able to see clouds of spores coming away from the gills. Thereupon I went out to the wood-shed where the temperature was about 32° F. and, with the aid of the lamp, attempted to observe the escape of the spores from the fruit-bodies remaining on the log, but all in vain: no trace of spore-clouds could be seen. It was surmised that the inactivity of the fruit-bodies on the log was due simply to their low temperature.

At 9 P.M. on the same day (November 24) I gathered another fruit-body from the log. This fruit-body had a temperature of about 32° F. and, when tested with the lamp for the emission of spore-clouds, gave negative results. At 9 P.M. I therefore was in

[1] Records taken at the Midland Institute Observatory, Edgbaston, Birmingham.

[2] *Vide* these *Researches*, vol. ii, 1922, pp. 101–102.

FIG. 198.—*Pleurotus ostreatus.* Fruit-bodies growing on the trunk of the same Silver Maple (*Acer saccharinum*) as that shown in Fig. 197. Pilei fully expanded. Photographed at Ithaca, New York State, on October 25, 1911, by C. D. Learn. About $\frac{1}{2}$ natural size.

possession of two fruit-bodies of *Pleurotus ostreatus* gathered from the same log on the same day: one gathered at 1.30 P.M., at a temperature of 70° F., shedding spores vigorously, which we will call A, and another, just gathered, at a temperature of 32° F., not shedding spores at all, which we will call B. I now reversed the temperatures of A and B. A was set outside on a cold window-ledge where the temperature was 32° F., while B was brought into the warm room where the temperature was 70° F. After an hour and a half had passed, A was brought back into the room and both A and B were examined with the hand-lamp. A now proved to be entirely inactive, while B gave forth spore-clouds vigorously. This experiment and its results are embodied in the accompanying Table:

The Effect of High and Low Temperatures upon the Production of Spore-clouds from Pleurotus ostreatus.

Fruit-body	Original Temperature.	Remarks on the Fruit-body's Activity.	Temperature after 1 hour 30 minutes.	Remarks on the Fruit-body's Activity.
A	70° F.	Emitting spore-clouds	32° F.	No longer emitting spore-clouds
B	32° F.	Not emitting spore-clouds	70° F.	Emitting spore-clouds

The experiment just described clearly proves that in *Pleurotus ostreatus* spore-discharge goes on very vigorously at high temperatures, such as 70° F., but very slowly or not at all at very low temperatures, such as the freezing-point of water. Doubtless, under natural conditions in the open, the fruit-bodies of *P. ostreatus* exhaust their basidia more slowly and live for a longer time when the weather is persistently cold than when it is persistently warm.

November and December Field-observations.—The next series of observations shows that, in the late autumn, under natural conditions, the spore-discharge period of *Pleurotus ostreatus* may exceed a month.

On November 23, about four feet from the ground, on the moribund trunk of one of the severely trimmed Poplars in the garden, I found several *P. ostreatus* fruit-bodies (Fig. 199) which had attained scarcely a quarter of their full size and which, therefore,

had probably begun their development about a week before (November 16 ?). Owing to the fact that the tree stood between some wooden palings and a dense Privet hedge, the fruit-bodies were well protected from the wind.

On November 23, and again on November 25, using my naked eye in diffuse daylight, I was unable to detect any spore-clouds coming from the fruit-bodies.

On December 1, the fruit-bodies were about one-half grown and their upper surfaces beautifully blue. The day was warm with a 24-hour maximum temperature of 46° F. and a minimum of 40° F.[1] At 10.15 in the morning, accompanied by Mrs. J. P. Hillhouse, I visited the tree and, on looking carefully at the edge of the largest fruit-body against the dark background of the palings, distinctly saw spore-clouds passing away at intervals from beneath the pileus ; and Mrs. Hillhouse soon perceived them too. There could be no doubt that, owing to the mild weather, the fruit-body was very actively discharging its spores. Conditions

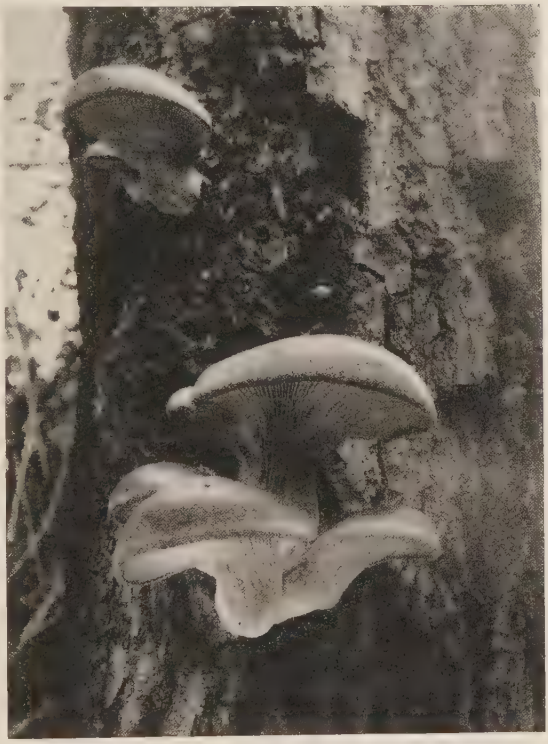


FIG. 199.—*Pleurotus ostreatus* on a dying Poplar (*Populus serotina*). Fruit-bodies grown to about one-third their full size, the pilei with blue upper surfaces and the extending pileus-margins still involute. Spores were seen escaping from these fruit-bodies by day on December 1 and December 15, and by night (by means of a hand-lamp) from December 15 to December 30. Photographed by J. P. Hillhouse at King's Heath, Birmingham, England, on November 25, 1922. About ½ natural size.

[1] Record of the Midland Institute Observatory, Edgbaston, Birmingham.

favouring the visibility of the spore-clouds, other than the high temperature, were: the stillness of the air, the protection from wind provided by the palings and the hedge, and the dark background which happened to be available.

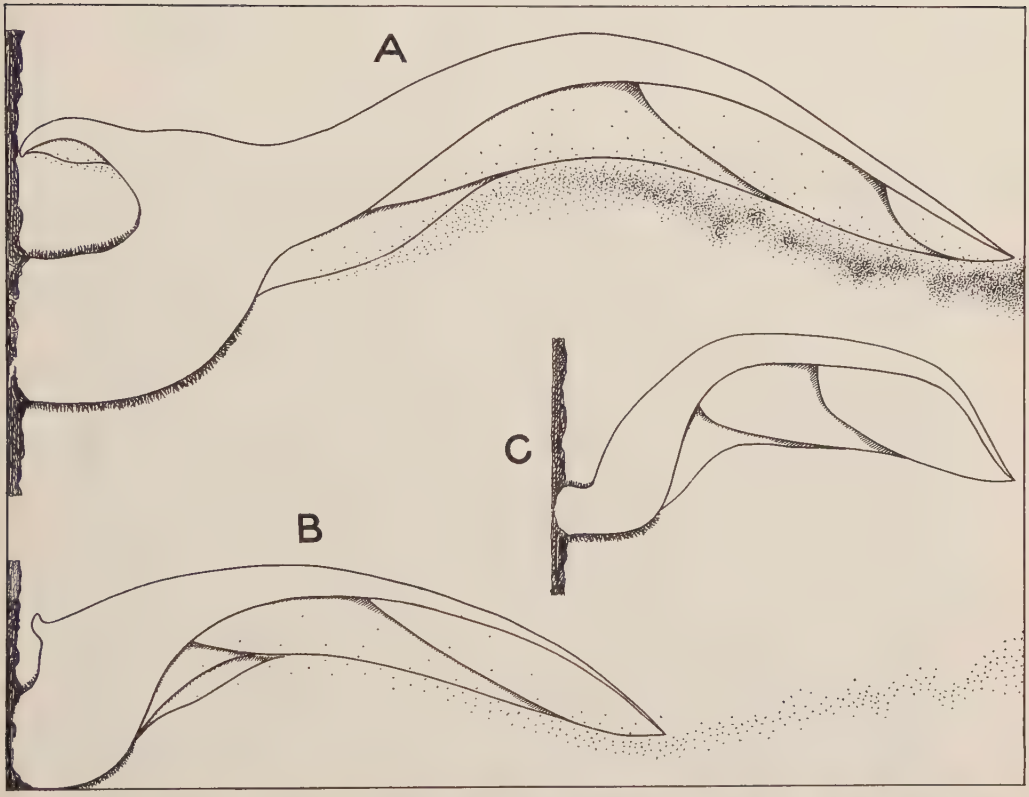
Absence from King's Heath prevented my making any observations on the fruit-bodies during December 2–9. On visiting the tree in the mornings of December 10–14, I was unable, using my naked eye in diffuse daylight, to observe any spore-clouds passing away from the fruit-bodies. This was doubtless due to low temperature or too strong a wind, or to both conditions combined.

December 15 was another warm and still day. On looking at the fruit-bodies at 3.30 P.M. when the temperature of the garden was 48° F., I again saw the largest one visibly shedding its spores. Relatively dense spore-clouds were passing from beneath the gills and becoming scattered by the wind (Fig. 200, A).

On the night of December 15, and nightly until December 30, I examined the largest fruit-body for the emission of spore-clouds. The electric hand-lamp, with which we have already become acquainted, provided a source of light; and, at the time of each observation, the temperature of the air was taken by means of a thermometer hung on a twig close by the fruit-body. On still cold nights, the air under the trees by the fruit-body was found to be several degrees warmer than that just above the grass of a near-by lawn. I hoped that, for some of the observations, it would cool down to the freezing-point of water, but its temperature was never once lower than 34° F.

At 11.45 P.M. on December 15, I went into the garden and, using the hand-lamp for the first time, examined the fruit-body. The weather was mild and the thermometer registered 43° F. Thick clouds of spores could be seen coming from the pileus and being borne away by the light airs of the night (*cf.* Fig. 200, D).

From December 15 to December 29, during fifteen successive nights, the fruit-body was found to be discharging spore-clouds. The coldest night was December 27 when the thermometer registered 34° F.; but, even at this temperature, the fruit-body continued to emit spores, although but relatively few per minute (*cf.* Fig. 200, B). On the night of December 29, which happened to be very mild, I was able to demonstrate the discharge of spores to two friends.



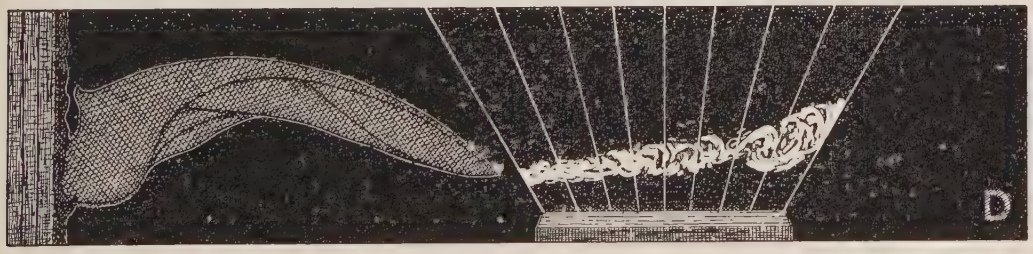


FIG. 200.—The emission of spore-clouds by *Pleurotus ostreatus*. Fruit-bodies attached laterally to the trunk of a Poplar, shown in section. A, B, and C illustrate the effect of temperature on spore-discharge: A, at 48° F., liberating spores vigorously; B, at 36° F., liberating spores feebly; C, at 32° F. (freezing-point), not liberating spores at all. D, a diagram showing how the electric hand-lamp was employed to observe spore-discharge at night. On the left a fruit-body, attached to a tree, emitting vast numbers of spores in the manner shown at A above. The spores are revealed as spore-clouds by means of the beam of light directed vertically upwards from the hand-lamp. The observer looks across the beam and sees the white clouds against the black shadows of the night. The observations on spore-discharge represented in A, B, and C were made at night with the hand-lamp. The emission of spores from A was also observed during the day in strong diffused sunlight. A, B, and C, natural size; D, reduced to about $\frac{3}{5}$.

About the middle of December, the fruit-body attained full size and, on December 18, its pileus measured 7 inches across from side to side and 5 inches from back to front. The blue colour of the

FIG. 201.—*Pleurotus ostreatus*. The under surface of a full-grown fruit-body removed from a Poplar (*Populus serotina*), showing the gills and the interlamellar spaces. Photographed by J. P. Hillhouse at King's Heath, Birmingham, England, on December 23, 1922. Natural size.

upper surface of the pileus had now disappeared and become replaced by a yellowish-brown. Moreover, the edge of the pileus, which during the first few weeks of the fruit-body's development had been characteristically involute (*cf.* Fig. 199, also Vol. I, Fig. 2, p. 22), had now become flattened or even somewhat turned-up (*cf.* Fig. 201, also Vol. I, Fig. 3, p. 23). A section

through the fruit-body made on December 30 is shown in Fig. 200 at A.

The data for the whole series of observations are embodied in the accompanying Table. In the fourth column, for the first four temperatures I am indebted to the records of the Midland Institute Observatory, made at Edgbaston, Birmingham. The remaining temperatures were all taken with my own thermometer set close to the fungus. In the fifth column the terms *very cold* (34°–35·5° F.), *cold* (36°–40° F.), *mild* (41°–45° F.) and *warm* (46°–50° F.) are, of course, merely relative.

Observations on the Production of Spore-clouds by a large Pleurotus ostreatus Fruit-body growing under Natural Conditions.

Date.	Source of Light.	Time of Observation.	Temperature at Time of Observation, Fahrenheit scale.	Weather.	Were Spore-clouds observed?	General Remarks from Field Note-book.
November 16	Sun	—	45°–33°	Mild .	—	About this date the fruit-body began to develop
November 23	,,	Morning .	50°–42°	Warm .	No	Fruit-body first observed
November 25	,,	Noon .	40° (?)	Cold .	No	Fruit-body about ½ grown. Edge of pileus incurved
December 1	,,	10.15 A.M.	43° (?)	Mild .	Yes	Air still. First observation of spore-clouds by daylight
December 15	,,	3.30 P.M.	48°	Warm .	,,	Last observation of spore-clouds by daylight. Spore-clouds dense
December 15	Lamp	11.45 P.M.	43°	Mild .	,,	First observation at night with a lamp. Spore clouds dense
December 16	,,	10.15 P.M.	42°	Mild .	,,	Spore-clouds carried away rapidly by the wind
December 17	,,	10.30 P.M.	42°	Mild .	,,	Wet. Spore-clouds observed with difficulty
December 18	,,	Midnight	40°	Cold .	,,	Spore-clouds carried away rapidly by wind. Fruit-body 7 × 5 inches, now full-grown. Edge of pileus turned up slightly

[*Continued on next page.*

Observations on the Production of Spore-clouds by a large Pleurotus ostreatus Fruit-body growing under Natural Conditions.—cont.

Date.	Source of Light.	Time of Observation.	Temperature at Time of Observation, Fahrenheit scale.	Weather.	Were Spore-clouds observed?	General Remarks from Field Note-book.
December 19	Lamp	5.45 P.M.	37°	Cold .	Yes	Spore-clouds light
20	,,	11.30–12 midnight	36°	Cold .	,,	
21	,,	9 P.M. (?)	46°	Warm .	,,	Windy and raining. Spore-clouds heavier than yesterday
22	,,	11 P.M. .	42°	Mild .	,,	Spore-clouds light
23	,,	7 P.M. .	41·5°	Mild .	,,	Rather gusty. Spore-clouds seen at intervals
24	,,	10.30 P.M.	35°	Very cold	,,	Light spore-clouds clearly seen
25	,,	11.30 P.M.	39°	Cold .	,,	
26	,,	6 P.M. .	37°	Cold .	,,	
	,,	9.30 P.M.	35°	Very cold	,,	Hoar-frost on grass. Light spore-clouds clearly seen
	,,	11.45 P.M.	35·5°	Very cold	,,	
27	,,	5.45 P.M.	34·5°	Very cold	,,	Hoar-frost on grass. Spore-clouds very light but unmistakable
	,,	10.20 P.M.	34°	Very cold	,,	
28	,,	5.45 P.M.	36·5°	Cold .	,,	Snow on lawn. Ice-crystals in field. Light spore-clouds clearly seen
29	,,	11 P.M. .	47·5°	Warm .	,,	Very wet. Spore-clouds relatively dense, demonstrated to friends
30	,,	12.10 A.M.	47·5°	Warm .	,,	

As shown by the Table, the fruit-body was observed shedding spores from December 1 to December 30. On December 30 I sailed for Canada, in consequence of which on that day my observations were brought to a conclusion. In all probability the fruit-body actually shed spores for some days prior to December 1 and for some days after December 30, although no observations were made during these periods. We may therefore conclude that, whereas the duration of the spore-fall period actually observed was four weeks and one day, the duration of the full spore-fall period must have been at least five weeks. Excepting possibly during a few brief hours when the night air cooled to about the freezing-point of

water, the fruit-body doubtless shed spores throughout its long-continued spore-discharge period continuously, both day and night.

It is interesting to note that the fruit-body continued to liberate spores, although feebly, at a temperature so low as 34° F., at a time when hoar-frost was forming on a near-by lawn ; and it is probable that clouds of spores coming from the pileus mingled with the snow that was falling on December 28. Evidently *Pleurotus ostreatus*, during a mild English winter, may shed spores even on Christmas Day and New Year's Day. Very rightly, therefore, it is described by English mycologists as " a fungus of the late autumn or winter."

Collybia velutipes and its Activity in Mid-winter.—*Collybia velutipes*, the *Velvet-stemmed Collybia*, with its viscid tawny pileus and dark velvety stipe, is one of the most beautiful of British Agaricineae (Fig. 202, also Vol. II, Fig. 15, p. 47). It usually occurs in dense clusters on logs and trunks of deciduous trees and is remarkable in that it has considerable powers of resisting frost. *Par excellence* it is a fungus of the winter months, but it also occurs in summer. Berkeley[1] has thus described it: "Pileus thin, fleshy, convex then plane, obtuse, smooth, viscid ; stem stuffed, velvety, rooting, dark bay ; gills adnexed, distant, yellowish."

Collybia velutipes also occurs in North America and I have often seen it at Winnipeg growing from the bark of dead or dying elms. Stewart,[2] who has made a special study of it as it occurs in New York State, remarks that it has been called the *Winter Mushroom* because of its ability to endure low temperatures. In his locality its principal seasons are October–November and May, but it may also be found in spells of mild weather throughout the winter. Stewart observed the fungus on Elm, Willow, Beech, Basswood, and Sugar Maple, but never on Conifers, and he considers that it shows a preference for Elm and Willow.[3] Among Stewart's other remarks on the biology of *C. velutipes* are the following :

" The favourite place of growth is on stumps at or near the soil line ; but clusters are found emerging from cracks at a considerable

[1] M. J. Berkeley, *Outlines of British Fungology*, London, 1860, p. 116.

[2] F. C. Stewart, *The Velvet-stemmed Collybia—a Wild Winter Mushroom*, New York Agricultural Experiment Station, Bull. no. 448, 1918, p. 86.

[3] *Ibid.*, p. 85.

distance above the ground. On the standing trunks of dead and dying trees, particularly elms in damp situations, the fungus may be found at a height of several feet . . . Partially buried wood and the partially exposed surface roots of stumps and dead trees are common places of growth. . . . *C. velutipes* prefers wood in a com-

Fig. 202.—*Collybia velutipes*. A cluster of fruit-bodies with dark velvety stipes, removed from the stump of a Maple and viewed somewhat from below. Collected at Ottawa, Canada, by W. S. Odell. Photographed by the Photographic Division of the Canadian Geological Survey. Natural size.

paratively early stage of decay. It is of little use to look for it on stumps and logs in an advanced stage of decay. The fungus is to be sought in new stump lots rather than in old ones. A stump may bear several crops each year for several years in succession and then cease to bear altogether." [1]

"The preference shown by *Collybia velutipes* for the wood of newly killed trees is in harmony with its semi-parasitic habit. The

[1] F. C. Stewart, *loc. cit.*, p. 85.

fungus is frequently found on the trunks of living trees, particularly those of the elm and willow, and has often been suspected of being parasitic under certain conditions.[1] Münch[2] and others have reported successful infection experiments with it." [3]

" With us it is the only wild mushroom obtainable in quantity during the winter. It may be frozen solid for days without affecting its quality or its ability to resume growth upon the return of mild weather. In fact it may freeze and thaw several times without material injury to its esculent qualities. . . . Apparently the plant is capable of growth at temperatures slightly above freezing. Probably it grows at any season when the ground is not frozen." On one occasion careful observation led to the conclusion that " the Collybia plants must have grown under the snow during the winter." [4]

" Compared with some of the common warm-weather mushrooms *Collybia velutipes* is of slow growth. . . . Caps of *C. velutipes* which have become shrivelled during dry weather absorb water and partially revive when moistened." [5]

" Its flavour is excellent. Most mushroom eaters of the writer's acquaintance pronounce it first class . . . The caps are firm, but never tough no matter how old they may be. When properly cooked they are palatable and easily digestible . . . Since they appear in cool weather, they are not seriously infested with worms or insects. . . . Another desirable quality . . . is the long time it may be kept." [6]

Collybia velutipes was one of the wood-destroying fungi which, during the winter of 1922–1923, were attacking the severely trimmed Poplars in my father's grounds at King's Heath, near Birmingham, England. A cluster of its fruit-bodies was observed in course of development on December 16, and at 7 P.M. on the night of December 23 I examined it with the hand-lamp. The temperature of the air was 41·5° F. The fruit-bodies were situated on the side

[1] P. Hennings, " Die an Baumstämmen und Holz auftretenden teilweise parasitaren heimischen Blätterschwämme," *Zeit. f. Pflanzenkrankheiten*, Bd. XIII, 1903, p. 204.

[2] E. Münch, " Versuche über Baumkrankheiten," *Naturw. Zeitschr. f. Forst- u. Landw.*, Bd. VIII, 1910, pp. 389–408, 425–447.

[3] F. C. Stewart, *loc. cit.*, pp. 86–87.

[4] *Ibid.*, pp. 86–87.

[5] *Ibid.*, pp. 88, 95–97.

[6] *Ibid.*, pp. 88–89.

of one of the Poplar trunks at a height of about 4 feet from the ground and, as the tree was somewhat exposed, to make successful observations I found it necessary to shield the fruit-bodies from the wind with my overcoat. On directing the beam of light horizontally in front of the group of fruit-bodies and looking across the beam vertically downwards or somewhat obliquely toward the ground I distinctly saw clouds passing through the light and being broken up and dispersed by air-currents. From December 24 to December 28 I visited the fruit-bodies nightly and, on each occasion, the lamp revealed clouds of spores coming away from the pilei. Preparations for my departure from England on December 30 then brought the observations to a close and I was therefore unable to determine the exact length of the spore-discharge period of the fruit-bodies investigated.

The observations just recorded show that the fruit-bodies of *Collybia velutipes* which came under my observation were liberating spores for six days. As in the investigation of *Pleurotus ostreatus*, it was found that spore-discharge was more active on warm nights than on cold, and that when the air was still it was much easier to detect the spore-clouds than when there was a decided breeze. The temperature for each observation was taken with a thermometer set near the fruit-bodies, and the thermometric data were practically identical with those already given for December 23–28 in the Table on p. 488. The lowest temperature for any observation was 34° F. on Christmas Eve; but, even on that occasion, the fruit-bodies continued to produce spore-clouds, although relatively thin ones.

From the hand-lamp observations just communicated we may conclude that the fruit-bodies of *Collybia velutipes*, like those of *Pleurotus ostreatus*, are so organised that, even when the temperature of the air is only a little above the freezing-point of water, they may freely carry out their one great function of producing and liberating spores.

It used to be thought that fungi necessarily cease all growth activity at, or a little above, the freezing-point of water. However, we now know that this is not always the case; for, in 1909, I [1] showed that the fruit-bodies of *Daedalea unicolor*, *Lenzites betulina*,

[1] A. H. R. Buller, these *Researches*, vol. i, 1909, pp. 124–126.

Polystictus versicolor, and *P. hirsutus*, all continue to develop and discharge their spores at 0° C., while Brooks and Hansford [1] have recently demonstrated that several moulds, such as *Cladosporium herbarum*—the *Black Spot Fungus*, *Torula botryoides*, *Sporotrichum carnis*, *Penicillium expansum*, and *Thamnidium* spp., which live on frozen meat and cause its deterioration, are able to grow at a temperature of from —6° to 0° C.[2] Since, as we have seen above, *Collybia velutipes* produces thin spore-clouds at a temperature of 34° F., it is possible, although no experiment has yet been made to decide the matter, that its basidia continue to develop and discharge spores very slowly even when the air by which they are surrounded has been cooled to 32° F.

[1] F. T. Brooks and C. G. Hansford, "Mould Growths upon Cold-store Meat," *Trans. Brit. Myc. Soc.*, vol. viii, 1923, pp. 113–141. These authors in their *Summary* say: "Some strains of *Cladosporium herbarum* will develop from spores at a temperature of — 6° C. and will give rise to considerable growths, including conidiophores, under prolonged cold-storage conditions. *Torula botryoides*, *Sporotrichum carnis*, *Penicillium expansum*, and *Thamnidium* spp. sometimes develop slightly at this temperature, but readily at 0° C., and it is probable that they grow appreciably between these two temperatures." For a previous report on the conditions of growth of the *Black Spot Fungus*, *vide* F. T. Brooks and M. N. Kidd, *Black Spot of Chilled and Frozen Meat*, Special report 6, Food Investigation Board, Dept. Sci. and Ind. Research, London, 1921.

[2] A French worker, C. Bidault, who has studied the moulds occurring on frozen meat, states ("Sur les Moisissures des Viandes Congelées," *Comp. rend. soc. biol.*, T. LXXXV, 1921, p. 1017), without giving details of his experiments, that *Chaetostylum Fresenii* (= *Thamnidium chaetocladioides*) and *Hormodendron cladosporioides* (= *Cladosporium herbarum*) will grow slightly at — 10° C., and that other species will grow between — 6° C. and 0° C. Cited from the paper of Brooks and Hansford *loc. cit.*, p. 114.

PART II

THE PRODUCTION AND LIBERATION OF BASIDIOSPORES IN THE UREDINEAE

CHAPTER I

THE PHENOMENA OF SPORE-DISCHARGE

Upon the Identity of the Phenomena of Spore-discharge in the Hymenomycetes and the Uredineae—The Discharge of Basidiospores in *Puccinia graminis*—The Discharge of Basidiospores in *Endophyllum Euphorbiae-sylvaticae*—The Discharge of Basidiospores in *Gymnosporangium Juniperi-virginianae*—Models of Basidia

Upon the Identity of the Phenomena of Spore-discharge in the Hymenomycetes and the Uredineae.—After Tulasne had discovered that the teleutospores of the Uredineae, on germination, produce a septate promycelium bearing four sporidia, and that the basidium in the Tremellineae is septate, it became obvious that the promycelium of the Uredineae is homologous with the septate basidium of the Tremellineae and with the non-septate basidium of the Hymenomycetes and Gastromycetes ;[1] and de Bary, who accepted the doctrine of evolution, expressed the view that the Uredineae, the Tremellineae, and the Hymenomycetes form a progressive phylogenetic series.[2]

The spore-bearing structure which is emitted by the teleutospore of a Rust fungus was called a *promycelium* by Tulasne and a *protobasidium* by Brefeld. In what follows we shall simply refer to it as a *basidium* which produces sterigmata and spores.

The view that the basidium is homologous in the Uredineae and Hymenomycetes was based in the first place on evidence of a

[1] L. R. et C. Tulasne, "Mémoire sur les Ustilaginées comparées aux Urédinées," *Ann. sci. nat.*, 3 sér., T. VII, 1847, pp. 12–127 ; "Seconde Mémoire," 4 sér., T. II, 1854, pp. 77–196 ; L. R. Tulasne, "Observations sur l'organisation des Trémellinées," 3 sér., T. XIX, 1853, pp. 193–231.

[2] A. de Bary, "Untersuchungen über die Peronosporeen und Saprolegnieen und die Grundlagen eines natürlichen Systems der Pilze," *Beiträge zur Morph. u. Phys. der Pilze*, Frankfurt a.M., 1881, p. 131, etc.

purely morphological nature. To its support, however, it is now possible to bring a number of physiological facts which have only recently been discovered. These concern the phenomena of spore-discharge, and will now occupy our attention.

In the Hymenomycetes it has already been shown that :

(*a*) From the point of view of structure :

(1) At the beginning of its development, a spore always forms a small projection called a hilum at the point where it joins the sterigma and on one side of it (Vol. II, Figs. 2 and 4, pp. 7 and 12).

(2) The axis of a spore taken through its point of attachment is always inclined to the axis of the sterigma (Vol. II, Figs. 2 and 4).

(*b*) From the point of view of function :

(3) The four spores leave the basidium in succession.[1]

(4) The spores are violently shot into the air to a distance of about 0·1–0·2 mm.[2]

(5) Some 5–15 seconds before a spore is shot away, a small water-drop always begins to be formed at the hilum of the spore.[3]

(6) As soon as the water-drop has attained a certain size, the spore is shot away along with the drop which clings to it.[4]

(7) After discharge of a spore, the sterigma does not immediately collapse, nor does it bear a drop of water or other substance at its summit.[5]

(8) The basidium-body does not collapse immediately as a result of the discharge of its spores.[6]

All these statements, with slight differences in the measurements given under (4) and (5), *are true also for the Uredineae.*

The basidiospores of the Uredineae, as compared with those of the Hymenomycetes, (1) are usually somewhat larger in size, and (2) are usually shot to a somewhat greater distance from their sterigmata (0·4–0·85 mm. in the Uredineae, as compared with 0·2–0·65 mm. in the Tremellineae, and 0·05–0·2 mm. in the

[1] These *Researches*, vol. i, 1909, pp. 133–147.
[2] *Ibid.*, p. 142.
[3] These *Researches*, vol. ii, 1922, pp. 8–17.
[4] *Ibid.*
[5] *Ibid.*
[6] *Ibid.*, pp. 7, 272, 355, 417.

non-tremelloid Hymenomycetes).[1] Moreover, (3) the water-drop excreted at the spore-hilum in the Uredineae is usually somewhat larger and takes a few seconds longer to attain maximum size than in the Hymenomycetes (10–40 seconds in the Uredineae as compared with 5–10 seconds in the Hymenomycetes).

So far as I am aware, Klebahn was the first to point out that in the Rust Fungi the spores are violently shot into the air. He says, in his work on Heteroecious Uredineae, published in 1904: "The sterigmata (Sporidienträger) appear to have the power to shoot away the sporidia a certain, although a very short, distance, so that it is not necessary for the wind first to break them off from the places of their formation." [2]

In 1912, Coons,[3] who employed the methods of observation described in the first volume of this work, proved conclusively that the sporidia of *Gymnosporangium Juniperi-virginianae* are violently shot away from their sterigmata into the air in the same manner as in Hymenomycetes. The horizontal distance of discharge was found to be 0·26–0·36 mm., and it was observed that the sterigma and the spore are both turgid immediately after spore-discharge.

In 1912, Dietel [4] published a paper on the discharge of sporidia in the Uredineae, in which he brought to light a number of new facts; and, upon reading it, I at once perceived that his observations exactly resembled my own made when studying the discharge of basidiospores in the Hymenomycetes. Dietel found that, when a teleutospore-sorus of Puccinia (*P. Malvacearum*, *P. Glechomatis*, *P. annularis*), Coleosporium (*C. Petasitidis*, *C. Campanulae*, etc.), or Cronartium (*C. asclepiadeum*) is cut out of a leaf and rested upon a drop of water under a bell-jar, the sporidia settle at a distance up to 0·6 mm. from the edge of the sorus, and that occasionally in Coleosporium, which has large spores, the distance measures 0·85 mm. Dietel came to the conclusion that the spores, under

[1] These *Researches*, vol. ii, 1922, p. 169.

[2] H. Klebahn, *Die wirtswechselnden Rostpilze*, Berlin, 1904, p. 30.

[3] G. H. Coons, "Some Investigations of the Cedar Rust Fungus, Gymnosporangium Juniperi-virginianae," *Annual Report* No. 25, Nebraska Agricultural Experiment Station, 1912, pp. 217–242.

[4] P. Dietel, "Über die Abschleuderung der Sporidien bei den Uredineen," *Mycologisches Centralblatt*, Bd. I, 1912, pp. 355–359.

normal conditions, can be shot about 0·6 mm. in a horizontal direction or about 0·3 mm. upwards. However, he was not satisfied with simply deducing the fact of violent spore-discharge from the position of the fallen spores, but went further and observed the discharge of spores from individual sterigmata. In *Coleosporium Petasitidis*, *Puccinia Arenariae*, and *Puccinia Malvacearum*, he discovered that, just before spore-discharge, a tiny drop of water appears at the top of each sterigma. His observations on the drop were as follows. The drop at the top of the sterigma grows rapidly and in Coleosporium attains a diameter of 9–10 μ in about 40 seconds. It often appears to push the spore a little to one side. When the drop has thus come into existence, the spore along with the drop is shot away from the sterigma with violence. If one places a cover-glass just above a teleutospore-sorus from which basidiospores are being discharged, the spores are shot up against the cover-glass and stick there along with the drop of water which each one has carried. Normal sporidia-discharge in the Uredineae, as thus described by Dietel, so closely resembles normal basidiospore-discharge in the Hymenomycetes, as described by myself, that doubtless the two processes are identical. Dietel believed that a difference existed between the Uredineae and the Hymenomycetes in that drops of water were excreted by the former just before spore-discharge and not by the latter. My account of spore-discharge in the Hymenomycetes had not then been published, so that it was left to me in 1915 to point out that this supposed difference does not exist and that *the phenomena accompanying the discharge of the basidiospores in the Uredineae and the Hymenomycetes are identical.*[1]

In 1917, Weimer,[2] using my beam-of-light method, like Coons, observed the fall of the basidiospores of *Gymnosporangium Juniperi-virginianae*. In one experiment the discharge of the spores continued from 6 P.M. until after 10 A.M. the next day. With the microscope, Weimer observed that the basidiospore farthest from

[1] A. H. R. Buller, "Die Erzeugung und Befreiung der Sporen bei Coprinus sterquilinus," *Jahrb. f. wiss. Bot.*, Bd. 56 (Pfeffer-Festschrift), 1915, pp. 314–315, 324.

[2] J. L. Weimer, "Three Cedar Rust Fungi. Their Life-histories and the Diseases they Produce," Cornell University Agri. Exp. Station, *Bull.* 390, 1917, pp. 523–524.

the teleutospore is the first to be formed and that the other three spores are formed in succession proceeding toward the basidium's base. Only one minute intervened between the disappearance of the apical basidiospore and its nearest neighbour, but a much longer period elapsed before the last two spores were discharged. Weimer states that " an abrupt sidewise movement of the basidiospore was always noticed several seconds previous to its discharge, and almost simultaneously a bubble appeared at its base." The " bubble " which he saw arise at the base of each spore just before discharge was undoubtedly a watery drop excreted from the spore-hilum. The " abrupt sidewise movement " of the spore may have been an optical illusion arising from the rapid development of the liquid drop. I myself have not observed such a movement in connection with the normal discharge of basidiospores either in the Hymenomycetes or in the Uredineae.

Dietel confined his investigations to species of Uredineae in which the teleutospores are capable of germination immediately after their formation, and he expressed the view that the spore-discharge phenomena might be somewhat different in species in which the teleutospores require a winter resting-period before they can put forth their basidia. In order to extend observations on spore-discharge to teleutospores which do not germinate until spring, I have examined *Puccinia graminis*. I have found that the phenomena of spore-discharge in this species are similar to those described by Dietel for other species of Puccinia in which the teleutospores germinate immediately. An account of my observations on *Puccinia graminis* will now be given.[1]

The Discharge of Basidiospores in Puccinia graminis.—At the beginning of May, 1914, on the bank of the Red River at

[1] I read a paper entitled : *On Spore-Discharge in the Uredineae and the Hymenomycetes* at Manchester on September 10, 1915, before the Botany Section of the British Association for the Advancement of Science (abstract in *Report of Bri. Ass.*, 1915, p. 730). I also read a paper entitled : *Some Comparative Observations upon the Shape of the Basidia and the Mode of Spore-Discharge in the Uredineae and the Hymenomycetes* before the Botanical Society of America at Pittsburg, on December 29, 1917, and again before the Canadian Branch of the American Phytopathological Society at Guelph on December 12, 1919 (abstract in *Phytopathology*, vol. x, 1920. p. 315). This second paper was illustrated with lantern slides and with the large models of straight and curved basidia described at the end of this Chapter.

Winnipeg, I gathered some haulms of the cultivated Oat (*Avena sativa*) which had been produced in the previous summer and which bore a large number of teleutospore-sori of *Puccinia graminis*. The Oat plants had been exposed to the weather during the whole of a long and severe winter, such as is normal for Manitoba. The temperature had often remained for periods of several days below 0° F. and had sometimes descended to between −30° and −40° F. Owing to the persistently low temperature during the winter and to the subsequent dry weather of the spring, none of the teleutospores had germinated. In order to provide conditions for germination, pieces of Oat stems bearing teleutospore-sori were placed so that they floated on the surface of drops of distilled water set on glass slides. The slides were then enclosed within a Petri dish. The teleutospores were by this means supplied with water without being submerged. As a rule the preparations were made in the evening. During the ensuing night the teleutospores germinated, and in the morning an abundance of basidia with sterigmata and basidiospores could be observed. The teleutospore-sori, after germination had set in, were examined under the low and the high powers of the microscope. Due care was taken to prevent rapid evaporation from the basidia by surrounding each preparation with a glass ring and by covering in the space between the top of the ring and the sides of the objective of the microscope with moist blotting paper.

The teleutospore of *Puccinia graminis* is two-celled, and each of the cells can give rise to a basidium. A basidium, as it grows outwards from the teleutospore-sorus, elongates at its apex and becomes curved at its free end. The free curved end of a basidium becomes divided by septa into four cells, which are more or less equal in size and contain approximately equal amounts of protoplasm. Each cell develops a sterigma and a single spore. The four spores of a single basidium usually develop simultaneously. A spore begins its development in the same manner as that of one of the Hymenomycetes, *i.e.* a hilum is first developed and the body of the spore takes up a lateral position at the end of the sterigma (Fig. 203, *a*, *b*, *c*, *d*). After a spore has attained its full size, all the protoplasm of the cell with which its sterigma is connected (presumably with the exception of a very thin lining layer) creeps

into the spore, so that, in the course of a few minutes, the cell becomes practically empty and the spore laden with protoplasm. The four septa doubtless strengthen the basidium mechanically, but I suspect that they have another and a more important physiological function: possibly by dividing up the long tubular end of the basidium into four equal cells they make it simpler for the protoplasm to be evenly distributed among the spores.[1] The appearance of a teleutospore with two fully developed basidia, one bearing four full-grown spores and the other with its spores in course of discharge, is shown in Fig. 204 at A.[2]

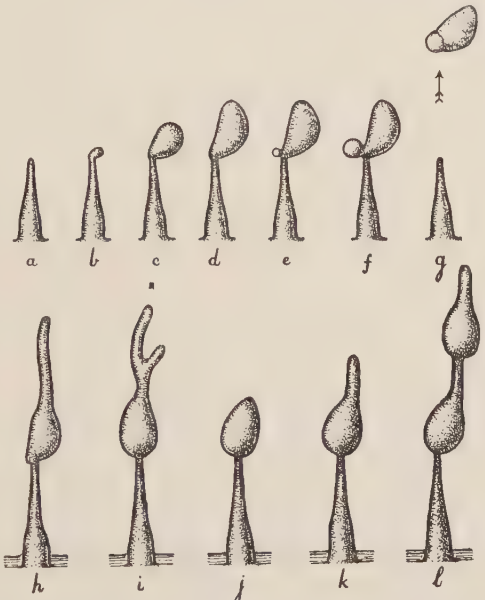


Fig. 203.—*Puccinia graminis.* *a* to *g*, normal development of a basidiospore and its discharge from the sterigma. *a*, the sterigma; *b*, the first rudiment of the spore, a hilum has been developed; *c*, the spore in a lateral position, half-grown; *d*, the spore full-grown. Stage *d* is about 20 minutes after stage *a*. Between *d* and *e* there is a pause of the order of an hour. *e*, a drop of water is beginning to appear at the hilum; *f*, five seconds later, the drop has attained its maximum size. The spore is then immediately shot away as shown at *g*. *h* to *l*, abnormal germination of undischarged spores on the ends of their sterigmata. The sterigmata have grown into the air from submerged basidia. *h*, a spore with an unbranched germ-tube; *i*, a spore with a branched germ-tube. The spore shown at *j* germinated, as shown at *k*. Its germ-tube produced another spore which germinated, *l*. Magnification, 880.

The development of the basidia and the formation of spores takes place a few hours after the teleutospores have been moistened. The rate of development of a spore on the end of its sterigma was carefully observed several times. Growth of a spore from its

[1] *Cf.* what was said concerning the septation of the basidia of the Auricularieae in vol. ii, 1922, pp. 165–166.

[2] Fig. 204 is semi-diagrammatic. Living basidia of *P. graminis* are often much more sharply curved than those there represented. The significance of basidial curvature, which was not fully realised when Fig. 204 was drawn, is treated of in the next Chapter.

first beginning as a tiny rudiment to full size was found to take from 15 to 25 minutes : the average time was 20 minutes (Fig. 203, *a–d*). In the Hymenomycetes, at least in some species, the growth period for the spores is practically identical with that for *Puccinia graminis*. Thus in *Collybia velutipes* the period was observed to be about 15 minutes, in *Armillaria mellea* 20 minutes, and in *Psalliota campestris* 40 minutes.[1] After attaining full size, a spore ripens : its full allotment of protoplasm passes into it through the sterigma, and changes take place at the base of the hilum which enable discharge to be effected. The length of time taken for a full-grown spore to ripen was not measured, but may be of the order of one hour. A spore, as soon as it is ripe, is shot away from its sterigma to a distance of a few tenths of a mm.[2]

After spending a considerable amount of time in concentrating my attention on individual basidia, I succeeded in observing the discharge of a few spores from their sterigmata in detail. In one preparation, where a basidium projected into the air, I clearly perceived that a tiny drop of water began to be excreted at the hilum at the base of one of the spores (Fig. 203, *e*). The drop grew visibly in size, and in from 5 to 10 seconds it became a little wider than half the diameter of the spore. Then the spore was violently shot away from its sterigma and disappeared from view (Figs. 203, *f* and *g*, 204, A, *b* and *c*). The drop disappeared at the same time as the spore and was doubtless carried with it, as in the Hymenomycetes, for it was not left on the vacant sterigma. The sterigma did not immediately collapse but seemed to remain turgid (Fig. 204, *d*). I have very little doubt that this mode of discharge, which corresponds to that described by Dietel for other Uredineae and to that which I have already described for the Hymenomycetes

[1] These *Researches*, vol. ii, 1922, p. 54.

[2] The basidia of the grain rusts, *Puccinia graminis*, *P. dispersa*, and *P. glumarum*, represented by Jakob Eriksson in his *Fungoid Diseases of Agricultural Plants* (London, 1912, Figs. 42, 44, and 47, pp. 69, 74, and 80) are all abnormal in form. They are shown as chains of cells either breaking up directly into separate cells or as directly constricting off conidia. Sterigmata are absent and the conidia have no hilum. Possibly these abnormal basidia were developed under too moist conditions. Unfortunately, Eriksson's drawings of the basidia of *P. graminis* and *P. glumarum* have been reproduced in von Tuboeuf's coloured wall-diagrams, V and VI, designed for the instruction of students.

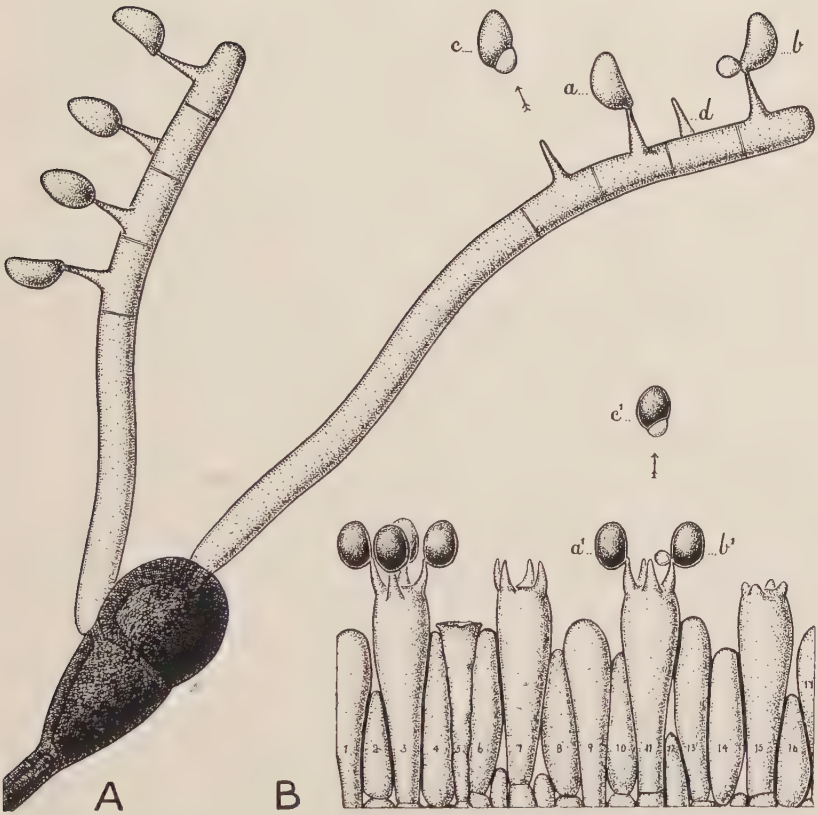


FIG. 204.—Comparison of spore-discharge in the Uredineae and the Hymenomycetes. A, a germinated teleutospore of *Puccinia graminis* (from *Avena sativa*) bearing two basidia, one with four ripe spores and the other with spore-discharge going on. At *a*, a spore as it appears about ten seconds before its discharge. At *b*, a spore at the instant before spore-discharge is effected : a drop of water has been excreted at the hilum and has attained full size. At *c*, a spore being shot away : it carries the drop with it. At *d*, a vacant sterigma which has not collapsed although its spore has been discharged some minutes previously. B, a transverse section through the hymenium of *Psalliota campestris* (wild form), showing a basidium with four ripe spores and another basidium with spore-discharge going on. The spores a^1, b^1, and c^1 correspond respectively to *a*, *b*, and *c* in A. In both *Puccinia graminis* and *Psalliota campestris* a drop of water is excreted from the hilum of each spore just before discharge. The elements represented in the hymenium of *Psalliota campestris* may be classified as follows : present-generation basidia, 3 and 11 ; past-generation basidia, 5 (now collapsed and drawn down); coming-generation basidia, 7 and 15 (about to develop spores); future-generations basidia, 1, 6, 9, 13, and 17 ; paraphyses, 2, 4, 8, 10, 12, 14, and 16. Magnification, the same for both A and B, 880.

generally, is normal for *Puccinia graminis*. In order that the striking resemblance of the modes of spore-discharge in the Uredineae and the Hymenomycetes may be clearly perceived, I have

represented semi-diagrammatically on the same scale in Fig. 204 a germinated teleutospore of *Puccinia graminis* and a portion of the hymenium of *Psalliota campestris*. On the left side of each drawing is shown a basidium bearing four ripe spores, and on the right side a basidium discharging its spores. The spores shown at *a*, *b*, and *c* in A correspond in their stages, in respect to discharge, to a^1, b^1, and c^1 in B.

The length of the sterigmata was found to be variable. In some basidia it was about 7 μ and in others as much as 18 μ.

In some of my preparations it was observed that the basidia had grown outwards from their teleutospores in a film of water on the glass slide and, whilst still submerged, had developed at the surface of the film sterigmata and spores, as represented in Fig. 205, A. The sterigmata always developed so that they grew into the air: it therefore seems likely that they are negatively hydrotropic. The spores developing on the ends of the sterigmata were normal in appearance; and, owing to their fixed position in space, their growth and discharge could be observed without much difficulty.

The discharge of spores developed from a submerged basidium, such as that represented in Fig. 205, A, was observed several times and was found to differ somewhat from the discharge of spores developed on aerial basidia, such as those represented in Fig. 204. Just as in an aerial basidium, in a submerged basidium a drop of water was excreted from the hilum before the discharge of each spore, but the drop often attained relatively gigantic proportions. A few minutes before discharge took place it was observed that a drop of water began to be excreted from the hilum and quickly attained a size equal to the maximum for spores borne on aerial basidia (Fig. 205, A, *a*). However, instead of the spore being discharged at this stage, the drop continued to grow, and soon it stepped up on to the body of the spore (Fig. 205, A, *b*). Further growth took place until the spore became entirely surrounded with a large drop from 10 μ to 17 μ in diameter. Then, after a delay of several minutes, the spore, surrounded by its drop, was suddenly shot away and became lost to view. In Fig. 205, A, there is shown at *c* a drop of water enclosing a spore which has just been discharged

and is travelling away from its sterigma, whilst at *d* is shown a drop of the maximum size observed, just prior to the discharge of itself and the spore contained within it. Some spores which excreted large drops of the kind just described were never shot away at all. Other spores, developed from submerged basidia, did not excrete drops at all and were not discharged. Several such spores were seen to germinate *in situ*, as shown in Fig. 203 at *h*, *i*, *j*, *k*, and *l* (p. 503). At *h* a spore on the top of its sterigma has developed an unbranched germ-tube : at *i* another spore has developed a branched germ-tube. The spore, *j*, germinated as shown at *k*, and then its germ-tube developed another spore which subsequently germinated as shown at *l*. The germination of certain spores *in situ* and the failure of some spores which have or have not excreted drops to be discharged are doubtless pathological phenomena. I consider that the excretion of the relatively enormous drops from submerged basidia in the manner just described is also pathological. It is to be doubted whether such conditions occur in nature as in my artificial preparations. Moreover, excessive excretion of water-drops is certainly a pathological phenomenon in a number of species of Hymenomycetes. We have already seen that a large drop of water was precociously excreted by a very immature spore of *Lepiota cepaestipes* (Vol. II, Fig. 9, p. 19) and that an excessive excretion of water sometimes takes place from the spores of *Coprinus sterquilinus* and *Psalliota campestris* at the time when the spores should be discharged.[1] For the sake of comparison with *Puccinia graminis*, the excessive excretion of water-drops by *Coprinus sterquilinus* and *Psalliota campestris* will now be described. In *Coprinus sterquilinus*, the normal size of the water-drop at the moment of discharge is that shown in Fig. 205, B, at the base of the right-hand spore. Occasionally, in some fruit-bodies which, perhaps, have been observed or have grown under unusually moist conditions, the drops continue growing and step up on to the body of the spores in the manner shown for the left-hand spore of B. The drop may then continue growing as shown at C ; and, finally, the spore with its abnormally large drop may be discharged as is indicated at C by means of the arrow. When the hymenium of a living gill

[1] *Cf.* vol. ii, 1922, pp. 17–18, 309.

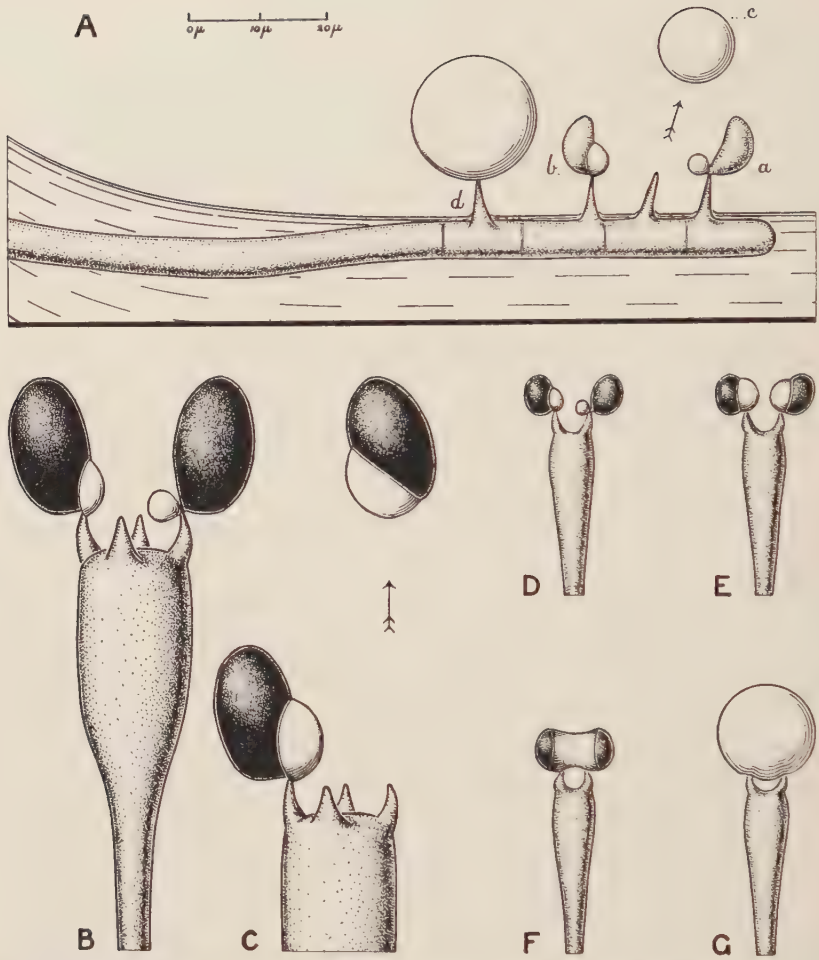


FIG. 205. Abnormal excretion of large drops from the hila of spores of Uredineae and Hymenomycetes. A, part of a basidium of *Puccinia graminis*, which has developed in a film of water but has produced aerial sterigmata and spores. At *a*, a drop of water about normal size has been excreted from the hilum. At *b*, a drop has grown beyond normal size and has stepped up on to the side of the spore. At *c*, a spore enveloped in a very large drop of water has just been shot from its sterigma. At *d*, a drop of the maximum size enclosing a spore. This globule was seen to be shot away. B and C, basidia of *Coprinus sterquilinus* with excretion of abnormally large drops of water before spore-discharge. B, the right-hand spore has excreted a drop of normal size. The drop on the left-hand spore has increased beyond the normal size and has stepped up on to the side of the spore. C, the drop on the left-hand spore has grown very large. The right-hand spore, bearing its abnormally large drop, has just been shot from its sterigma. D, E, F, and G, stages in the abnormal excretion of water from the hila of two spores of a basidium of the cultivated Mushroom (*Psalliota campestris*). D, one drop is of normal size, the other larger than normal. The latter has stepped up on to the side of the spore. E, the drops have grown larger. F, the drops have touched and joined, so as to form one drop which has dragged the spores toward one another. G, the drop has continued to increase in size and has now become globular. The spores were not discharged. Magnification, the same for all, 880. A scale is shown at the top of the Figure.

of *Psalliota campestris* is looked down upon through a cover-glass, one sometimes sees the drops begin their excretion normally, as shown for the right-hand spore in Fig. 205, D. Then the drops may step up and continue growing, as in the left-hand spore of D and in E. Growth may continue until the two drops touch and join, as at F. Further growth may result in the formation of a large globular drop on the tops of the two sterigmata, as shown at G. In *Psalliota campestris*, spores which excrete these abnormally large drops are never discharged. It seems clear that the abnormal excretion of drops from the hila of spores in the Hymenomycetes finds its parallel in the Uredineae, at any rate in *Puccinia graminis*.

From all the observations which have been recorded in the foregoing pages on *Puccinia graminis*, we may conclude that both the normal and the abnormal phenomena accompanying the discharge of the spores are similar to those which are characteristic of Hymenomycetes. The mechanism of spore-discharge in the Uredineae and the Hymenomycetes is doubtless identical. This deduction, from physiological studies, serves to strengthen the belief in the close genetic relationship of the two groups.

The nature of the force which brings about the discharge of the spores is just as much a mystery in the Uredineae as in the Hymenomycetes. That there is some powerful force which can be applied just beneath the hilum is shown by the fact that so relatively large a mass as that shown in Fig. 205, A, *d*, can be shot away a considerable fraction of a mm. from the top of the slender sterigma.

The Discharge of Basidiospores in Endophyllum Euphorbiae-sylvaticae.—The genus Endophyllum is unique among the Rust Fungi in that the aecidiospores, on germination, instead of producing a mycelium, at once give rise to basidia. It is believed by Grove [1] that the genus is a primitive one and that, during its phylogenetic development, it has never possessed a uredospore stage; but Dietel [2] and others look upon it as a product of reduction

[1] W. B. Grove, "The Evolution of the Higher Uredineae," *The New Phytologist*, vol. xii, 1913, p. 100.

[2] P. Dietel, in a review of W. B. Grove's "The British Rust Fungi," *Mycologisches Centralblatt*, Bd. IV, 1914, pp. 209–210.

and hold that its life-history is simply a shortened form of a Eu-puccinia such as *Puccinia Violae*. As possibly bearing on this question, it seemed to me of interest to determine whether or not the discharge of spores from the basidia of Endophyllum takes place in the manner which is normal for the other Uredineae as well as for the Hymenomycetes.

In June, 1915, Mr. W. B. Grove kindly procured for me from Credenhill Camp, Herefordshire, some shoots of the Wood Spurge (*Euphorbia amygdaloides*) which were strongly infected with *Endophyllum Euphorbiae-sylvaticae*. The aecidia were thickly set on the backs of the leaves (Fig. 206, A); and, when the shoots were shaken, the ripe aecidiospores fell out of their aecidia in the form of a fine powder.

The following method was employed in cultivating the aecidiospores. Some glass slides were smeared with a warm 10-per-cent. gelatine solution. As soon as the gelatine had cooled and set, an infected shoot of the Euphorbia was shaken over each slide. A light shower of aecidiospores thus came to settle on the surface of the gelatine. The slides were then put in Petri dishes, the bottoms of which had been covered with moistened blotting paper. The Petri dishes were kept at a room temperature of about 18° C. The cultures were made at 9 P.M. A considerable number of the aecidiospores germinated during the night, and the discharge of the basidiospores took place in abundance during the succeeding morning and afternoon.

Observations upon the germinated aecidiospores were carried out as follows. A slide was removed from a Petri dish, set on the stage of the microscope, and a favourable place in the preparation found as quickly as possible with the low-power objective. A glass ring, 22 mm. wide and 2 mm. high, was then rested on the slide so as to include the favourable place, the high power of the microscope was applied, and finally the space between the top of the glass ring and the objective was covered over with strips of wet blotting paper. Thus a small damp-chamber was constructed, and it became possible to look down upon a number of germinated aecidiospores and to observe their basidia whilst these were continuing their normal development.

An aecidiospore, on germinating, produces a short basidium about 50 μ long and 6 μ wide. Into this flows all the protoplasm contained in the aecidiospore. The passage of this substance can be readily followed owing to the fact that it has a yellow colour. Across the basidium-body three septa are formed which break it up into four cells of about equal size (Fig. 206, B, C). Each cell gives rise to a conical sterigma which is about 7 μ long. At the top of each sterigma a spore is developed in the usual manner, *i.e.* a hilum is first formed and then a spore-body, the latter growing in a direction which is inclined at an angle of about 30° to the axis of the sterigma (F, *a*). The spores are more or less pear-shaped but flattened or slightly concave on the side toward the hilum; and their length is 9–10 μ. The four spores of a basidium commence to

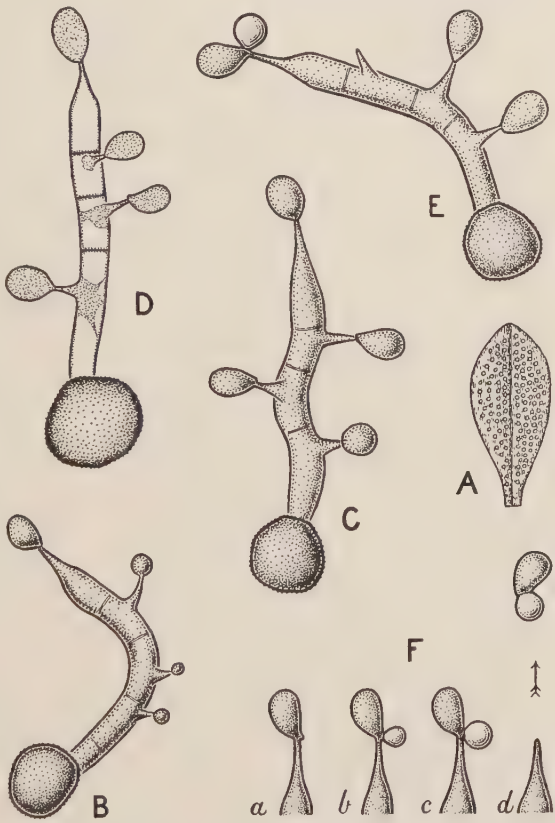


FIG. 206.—*Endophyllum Euphorbiae-sylvaticae.* Development and discharge of basidiospores. A, leaf of *Euphorbia amygdaloides* with numerous aecidia. B–E, aecidiospores which have germinated and have produced basidia. B, basidiospores developing, each one a few minutes old, the end one the oldest. C, all the basidiospores are full-grown. D, optical section through a basidium in which the protoplasm is passing up the sterigmata into the spores. E, a basidium discharging its spores; one spore has gone, and the end one with a drop of water excreted at its hilum is just about to be discharged. F, successive stages in the discharge of a single spore; *a*, sterigma and lateral view of a spore showing the hilum; *b*, a drop has been excreted from the hilum; *c*, the drop has grown to its maximum size; *d*, the spore with the drop clinging to it has just been discharged, leaving the sterigma uncollapsed. A, natural size; E–F, magnified 587.

develop about the same time, but it often happens that one or two, particularly those at the end, attain full size before their fellows (B). As a rule, however, the slightly younger spores of a basidium become full-sized before the slightly older ones are discharged (C).

The passage of the yellow protoplasm from the basidial cells into the basidiospores takes about an hour. Often from the clump of protoplasm passing into the funnel-shaped sterigma bridles pass to the walls of the cell concerned ; and one can also often detect the presence of tiny particles on the cell-walls from which the massive protoplasm has retired (D). These facts seem to indicate that a lining layer of protoplasm is left in the cells until after the discharge of the spores. There seems to me little doubt that not only is this lining layer present but that it is also of distinct importance in enabling the basidium to maintain its turgidity.

In order to observe the discharge of a spore when magnified about 450 times, it is necessary for one to concentrate one's attention on a spore which appears ripe, *i.e.* one which is of full size and packed with the yellow protoplasm, and which has an associated basidial cell emptied of massive contents (*e.g.* Fig. 206, C). After waiting some minutes or the greater part of an hour, one may at length see the event happen. Suddenly a drop of water begins to be excreted from the hilum of the spore : the drop quickly grows, and, in the course of 15–25 seconds, attains nearly the same diameter as that of the spore (F, *a*-*c* ; E). Then, in a flash, the spore is shot out of sight together with its drop (F, *d*). The sterigma is left vacant, but does not collapse : its end appears closed, at any rate one cannot detect a hole in it or any sign of its having been broken across. The spores are shot off one after the other in succession in the course of several minutes or an hour ; and, when all the spores have gone, the basidium collapses and dies. There is practically only an empty shell of chitin left behind : the living substance has all been sent away on four journeys through the air.

The distance to which the basidiospores can be shot from the basidia in a horizontal direction in still air can be found without much difficulty. In a preparation which has developed in a closed Petri dish one first finds places where the aecidiospores lie far apart.

Then it is safe to assume that the four basidiospores lying around one of these isolated aecidiospores have been discharged from its basidium. With the help of a micrometer the distances of the spores from their basidium can be at once measured. A number of such measurements were made, and it was found that the basidiospores had often travelled a horizontal distance of 0·2–0·3 mm. before settling. The maximum distances observed were rather less than 0·5 mm. This distance agrees in its order with that observed by Coons and Dietel for other Uredineae. Coons, it will be remembered, observed that the basidiospores of *Gymnosporangium Juniperi-virginianae* can be shot away 0·26–0·36 mm., while Dietel came to the conclusion that in several species of Puccinia, etc., the spores can be shot 0·6 mm. in a horizontal direction and 0·3 mm. upwards, and that in Coleosporium, which has large spores, the maximum horizontal distance may be as much as 0·85 mm.

A comparison of the observations made upon *Endophyllum Euphorbiae-sylvaticae* with those made upon *Puccinia graminis* and those to be described for *P. Malvacearum* has convinced me that there is nothing primitive about the structure of the basidium in Endophyllum or in the mode of spore-discharge. My investigations therefore support Dietel's view that Endophyllum has obtained its peculiar life-history by reduction rather than the view of Grove that this fungus is a primitive member of the Uredineae which has never developed typical teleutospores. I shall show, in connection with *Puccinia Malvacearum*, that the sickle shape of the basidium and the arrangement of the spores on the convex side, which characterise the basidia of Uredineae in general, are of high importance in permitting of the efficient discharge of the basidiospores from teleutospore-sori developed on stems and leaves, etc. The use of the curvature in the basidium is by no means so obvious when the basidium is developed from isolated aecidiospores, as is the case in Endophyllum. Yet the basidia in Endophyllum are frequently curved in the typical manner (Fig. 206, B, E), with the basidiospores arising on the convex side. I am inclined to think that this tendency to marked curvature in the basidium of Endophyllum has been inherited from Uredineae in which it was an important factor in securing the liberation of the basidiospores, *i.e.*

from Uredineae which possessed teleutospores that germinated in their place of development upon their host in the manner characteristic of normal Uredineae at the present day.

Fig. 207.—*Gymnosporangium Juniperi-virginianae* on *Juniperus Virginiana*. Mature reddish-brown Cedar apples, photographed April 2. The teleutospore-sori have just ruptured the cortex of the gall preparatory to the projection of gelatinous horns during the next rain. Photographed by H. S. Reed and C. H. Crabill. From *Bull.* No. 9 of the Virginia Agricultural Experiment Station. Natural size.

The Discharge of Basidiospores in Gymnosporangium Juniperi-virginianae.—*Gymnosporangium Juniperi-virginianae* is a heteroecious rust fungus which produces its teleutospores on the Red Cedar, *Juniperus virginiana*, and its aecidiospores on the Apple (species of Malus). When attacking the Red Cedar, the mycelium causes hypertrophy of individual leaves, so that each infected leaf develops into a large leathery gall known as a *Cedar apple* (Fig. 207).[1] The galls are often several centimetres in diameter and there may be scores or even hundreds of them present upon the twigs of a single tree.

In West Virginia, U.S.A., Cedar galls first become apparent during June, continue to grow through the summer, and almost reach maturity in late autumn. With the first warm weather of spring, they develop numerous brownish projections which are nothing more or less than

[1] H. S. Reed and C. H. Crabill, "The Cedar Rust Disease of Apples caused by Gymnosporangium Juniperi-virginianae," Virginia Agricultural Experiment Station, *Bull.* 170, 1915, p. 18, Fig. 4.

teleutospore-sori.[1] Each sorus is composed of many two-celled teleutospores embedded to a greater or less degree in a gelatinous matrix derived from the teleutospore-pedicels. Under favourable weather conditions, with sufficient moisture, the sori swell up into large reddish-brown gelatinous horns on the exterior of which are numerous teleutospores (*cf.* Figs. 207, 208, 209, and 210). Each of

FIG. 208.—*Gymnosporangium Juniperi-virginianae* on *Juniperus virginiana*. Two Cedar apples (rust galls) of medium size with expanded gelatinous teleutospore-sori. Photographed by N. J. Giddings. From *Bull.* No. 170, of the West Virginia Agricultural Experiment Station. About natural size.

the two cells of a teleutospore may send out a basidium (promycelium).[2] Each basidium becomes divided by septa into five cells, a sterile stalk-cell and four other cells each of which develops a sterigma and a basidiospore (Fig. 211).[3] As first observed by Coons,[4] the basidiospores, when ripe, are violently discharged from their sterigmata into the air.

[1] N. J. Giddings, "Infection and Immunity in Apple Rust," West Virginia Agricultural Experiment Station, *Bull.* 170, 1918, p. 9.

[2] *Ibid.* [3] H. S. Reed and C. H. Crabill, *loc. cit.*, p. 27, Fig. 8.

[4] G. H. Coons, "Some Investigations on the Cedar Rust Fungus, Gymnosporangium Juniperi-virginianae," *Annual Report* No. 25, Nebraska Agricultural Experiment Station, 1912, pp. 217–242.

Through the kindness of Dr. N. J. Giddings, on April 25, 1924, I received from West Virginia a packet of freshly gathered Juniper twigs bearing numerous Cedar apples. Some of these galls were soaked in water for 1 hour, some for 2 hours, and some for 3 hours, whilst others were sprayed with tap-water splashings for 2 hours. Whilst in contact with the water, the teleutosporic horns became greatly swollen (Fig. 209). After the moistening periods were over,

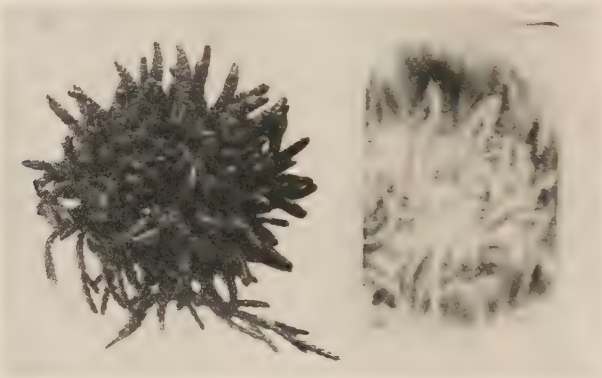


FIG. 209.—*Gymnosporangium Juniperi-virginianae* on *Juniperus virginiana*. Left, a Cedar apple, after absorption of water, with each teleutospore-sorus now extended into a long gelatinous horn bearing the teleutospores. West Virginia material received in Winnipeg, April 25, and then revived by soaking. Right, a spore-deposit of red basidiospores (sporidia) which accumulated in one night under the horns of a Cedar apple like that shown on the left. Natural size.

the galls were set in glass chambers. By the next morning all the galls had produced orange spore-deposits (Fig. 209), thus proving that the teleutospores had germinated and had given rise to normal basidia and basidiospores.

About six hours after some Cedar galls had been sprayed, I cut off a gelatinous horn from one of the galls, placed it in a glass-ring cell, covered the cell with a cover-glass, and then, using the low power of the microscope, observed what was happening. At once it could be seen that a considerable number of teleutospores had developed short basidia which were turned back toward the surface of the horn, and that the sterigmata and basidiospores were on the outer convex side of each basidium and were therefore directed

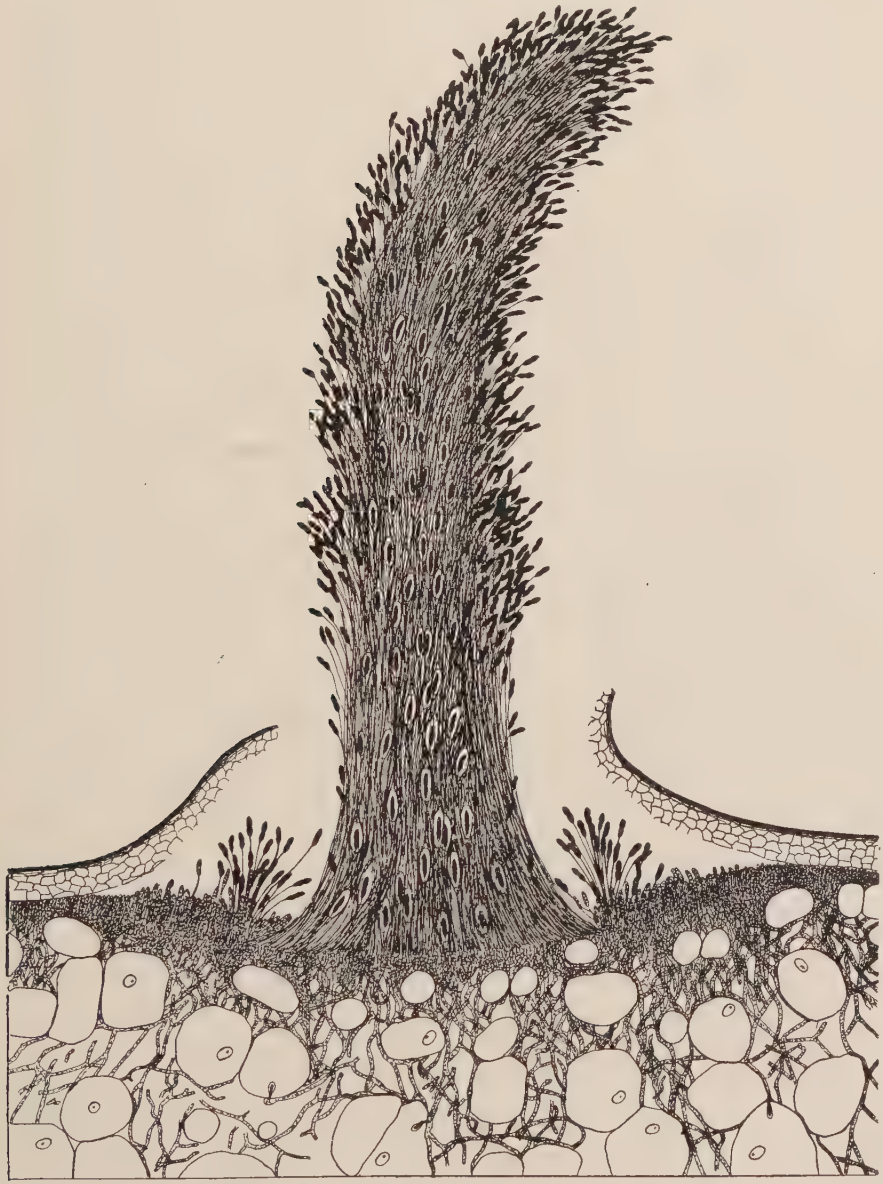


FIG. 210.—*Gymnosporangium Juniperi-virginianae* on *Juniperus virginiana*. A longitudinal section through a partly gelatinous teleutospore-sorus, after the first extrusion of horns in April. The teleutosporic horn has broken through the cortex of the Cedar apple. Drawn by H. S. Reed and C. H. Crabill. From *Bull.* No. 9 of the Virginia Agricultural Experiment Station.

away from the surface of the horn. On observing intently for a few minutes particular basidiospores which were of full size and apparently ripe, I was soon rewarded by seeing them violently discharged from their sterigmata, thus confirming the previous observations of Coons, Giddings and Berg, and Weimar.

FIG. 211.—*Gymnosporangium Juniperi-virginianae*. Teleutospores and their germination. From left to right: (1) a typical mature teleutospore, (2) a young teleutospore in which large oil drops are still present, (3) a one-celled teleutospore, (4) typical germination of a teleutospore, the upper cell having produced a curved basidium with four sterigmata and four spores on its outer convex side, and (5) a teleutospore showing abnormalities in the mode of germination, *viz.*, swelling of two germ-pits and production of germ-tubes directly from two of the basidial cells. Drawn by H. S. Reed and C. H. Crabill. From *Bull.* No. 9 of the Virginia Agricultural Experiment Station.

Whilst watching basidiospores about to be discharged, I concentrated my attention upon the spore-hilum, and I then perceived that a drop of water was excreted from each hilum immediately before the moment of discharge. The drop made its first appearance 10–40 seconds (often about 15 seconds) before discharge and grew in size to somewhat less than the spore's diameter. Then the drop and spore were violently shot away together, just as in *Puccinia*

graminis and *Endophyllum Euphorbiae-sylvaticae*. This phenomenon was demonstrated to a research-worker, Mr. W. F. Hanna, as follows. I observed a basidiospore intently and, as soon as a drop began to appear on the spore-hilum, I asked Mr. Hanna to look down the microscope. He did so and, in the course of about 10 seconds, saw the drop grow to full size and then the drop and spore violently discharged from the sterigma.

Each basidiospore is set obliquely on the end of its sterigma from the first, as in *Puccinia graminis* and the Uredineae in general. During the excretion of the drop, a basidiospore does not become pushed to one side, as believed by Weimar,[1] but remains unmoved.

Finally, it may be mentioned that the discharge of the basidiospores from Cedar galls can be very well seen, and demonstrated to students, by means of the beam-of-light method described in Volume I.[2] When an active gall is suspended at the top of a covered beaker and a beam of light is directed through the air just below the gelatinous horns, the individual basidiospores, as they are shot away from the horns and spring into the light, can be perceived quite readily as white particles falling by their own weight or being carried hither and thither by convection currents in the air.

Models of Basidia.—For use with audiences of students and others, I have had constructed two large models representing respectively the curved basidium of a Rust fungus (*Puccinia Malvacearum*) and the straight basidium of a Hymenomycete (generalised). These models are photographically reproduced in Fig. 212. The taller of the two is two feet high. In the Rust model (to the left) one sees a two-celled teleutospore bearing a curved basidium-body with four sterigmata and four spores. The model is detachable from its stand, the basidium detachable from the upper cell of the teleutospore, and the four spores detachable from their sterigmata. In the hymenomycetous model (to the right) one sees a straight basidium-body bearing four sterigmata and four spores. Again the model is detachable from its stand and

[1] J. L. Weimar, "Three Cedar Rust Fungi. Their Life-histories and the Diseases they Produce," Cornell University Agricultural Experiment Station, *Bull.* 390, 1917, pp. 523–524.

[2] These *Researches*, vol. i, 1909, pp. 94–101.

the four spores detachable from their sterigmata. Detachment of one part from another in each model is accomplished simply by a

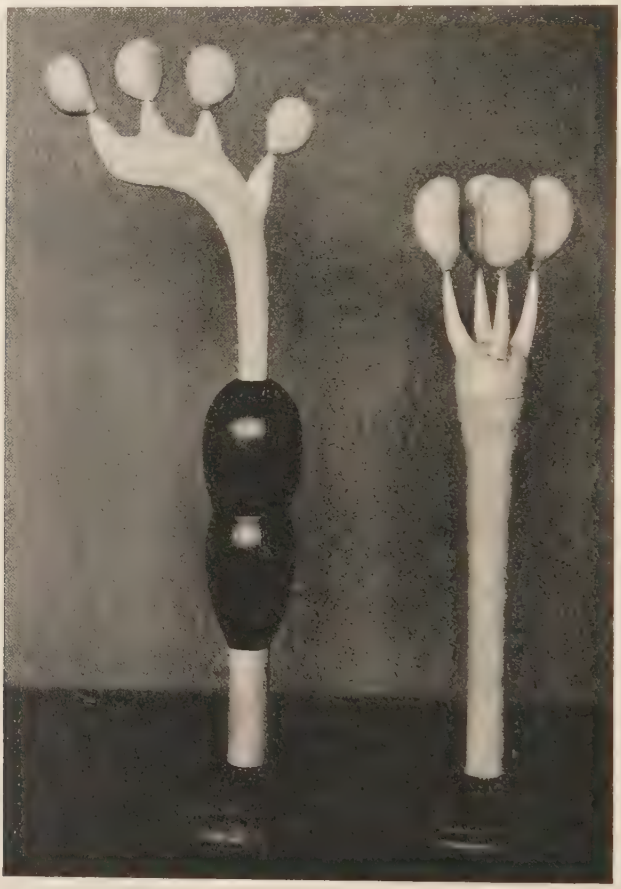


FIG. 212.—Two models of basidia, the taller two feet high. To the left, a teleutospore of a Rust fungus (*Puccinia Malvacearum*) from the upper cell of which projects a curved basidium with four sterigmata and four spores; the basidium detachable from the teleutospore, and the spores from the sterigmata. To the right, a basidium of a Hymenomycete, the spores detachable from the sterigmata. By holding each model horizontally with the left hand and by pulling off the spores one by one with the right hand, one can show to an audience the violent and successive discharge of the spores and the sporabolic trajectories.

gentle pull. The Rust model, made entirely of wood, was constructed at Winnipeg by my former laboratory attendant, Rudolf

Hiebert; and the model of the Hymenomycete, made of brass (top of basidium-body, sterigmata) and wood (stand, shaft of the basidium-body, spores), was constructed by Mr. Churchward at the University of Birmingham. The brass in the latter model was used instead of wood to prevent the slender sterigmata from breaking during demonstrations, transportation, etc.

With the Rust model, to show how the basidium develops, one first exhibits the teleutospore by itself, then adds to it the basidium-body and its four sterigmata, and then the four spores. Similarly, to show development with the hymenomycetous model, one first exhibits the basidium-body with its four naked sterigmata and then adds the spores.

In the Rust model each spore has a slight hilum at its base. This is where a drop of water should be excreted before discharge. If one takes a little ball—such as a ping-pong ball—and holds it at a hilum, one can indicate the position, general appearance, and size of the water-drop. In the hymenomycetous model, unfortunately, the spore-hila were not constructed.

To illustrate the phenomenon of spore-discharge in still air with the Rust model, one lifts the model from its stand, grasps the stalk of the teleutospore in the left hand, and holds the main axis of the model in a horizontal position. With the thumb and first finger of the right hand one then takes hold of one of the spores and, keeping hold of the spore, one makes it travel as rapidly as possible straight outwards from its sterigma (more or less horizontally) for a distance of about two feet, then turns it downwards sharply through a right angle, and finally makes it descend vertically downwards with a relatively slow terminal velocity without any acceleration. One then deals with the other three spores in exactly the same manner. At the end of these operations, the sterigmata are all vacant of spores and one has made the audience clearly visualise the violent mode of spore-discharge and the peculiar nature of the sporabolic trajectory. One then handles the hymenomycetous model in the same manner as the Rust model, and it at once becomes obvious that the phenomenon of violent spore-discharge in the Uredineae and in the Hymenomycetes is essentially similar.

CHAPTER II

THE TELEUTOSPORE AND THE CURVATURE OF THE BASIDIUM

A Comparison of the Mechanism for the Production and Liberation of Basidiospores in the Uredineae and the Hymenomycetes—The Shape and Position of the Basidia of *Puccinia Malvacearum* during Spore-discharge

A Comparison of the Mechanism for the Production and Liberation of Basidiospores in the Uredineae and the Hymenomycetes. – The Uredineae and the Hymenomycetes both belong to the great group Basidiomycetes because they both develop basidia and spores. The manner in which the Uredineae and the Hymenomycetes became differentiated from one another in the course of evolution is still a matter of controversy, and writers have constructed a number of phylogenetic trees corresponding to their various views; but it is generally agreed that in both groups the basidiospores are phylogenetically the most primitive organs of dissemination. It is further agreed that the conidia and chlamydospores which are produced in connection with the mycelium or the fruit-body of certain Hymenomycetes, and also the uredospores and aecidiospores which are produced by many Uredineae, are, relatively to the basidiospores, of later origin. It is possible that the uredospores and aecidiospores are modified teleutospores; but with these secondary organs of dissemination we shall not now concern ourselves. In what follows we shall confine our attention to the basidiospores.

The Uredineae are all parasites, entirely dependent on their host-plants. On the other hand, the Hymenomycetes, with comparatively rare exceptions, are saprophytes. It seems to me that it is this different mode of life which has led to the difference in the manner in which the basidiospores are produced in the two groups.

In the Hymenomycetes, the basidia arise upon a fruit-body of greater or less complexity and they lie with their axes parallel to one another in a compact layer called the *hymenium* which covers part of the outer surface of the fruit-body. Each basidium arises from a sterile subhymenial cell from which it is cut off at the moment of its formation; and, soon after its formation, under normal conditions of temperature and moisture, it produces and discharges its basidiospores. The basidium cannot be considered as a structure especially adapted to undergo a prolonged resting period: its cell-wall is very thin, it easily loses water, and, except for a number of species where the fruit-bodies usually grow on wood and where every cell of the fruit-body can survive desiccation, dies when it is dried. For instance, desiccation of the gills of a Mushroom, a Tricholoma, a Psathyra, or a Coprinus is fatal to every basidium which the gills contain. Moreover, a basidium does not become independent of the subhymenial cell from which it is derived but, until it has discharged its basidiospores, draws upon it for its supplies of moisture. It would be hopeless to try to isolate a basidium from the hymenium of one of the Hymenomycetes and expect it to continue to produce its sterigmata and spores.

In the Uredineae, on the other hand, there is no fruit-body comparable to that which occurs in the Hymenomycetes; and such a fruit-body is unnecessary on account of the fact that the Uredineae are parasites: the stems, leaves, or inflorescences of their hosts constitute admirable substrata upon the surfaces of which the basidia can be produced. The tissues of the host-plants take the place of the fleshy parts of hymenomycetous fruit-bodies, in that they supply the food and water necessary for the development of the basidia; whilst the position of the infected leaves, stems, etc., above the ground, is such that the basidiospores, when liberated, escape into the air in such a manner that they can readily be carried off by the wind. A structure like the stipe which characterises the fruit-bodies of so many terrestrial Hymenomycetes, and the chief function of which is to place the hymenium in such a position that when the spores are liberated from the basidia they will fall into an open space where the wind can carry them away, is therefore rendered unnecessary in the Uredineae.

In the Uredineae, a basidium does not arise directly from one of the hyphae which are to be found under the epidermis of the host-plant but indirectly, through the intermediary of a special body known as a *teleutospore*. A teleutospore is not an organ of dissemination like the aecidiospore, the uredospore, and the basidiospore ; but, as van Tieghem has pointed out, it is a *probasidium*, a cellular structure which is destined not to produce a new fungus plant, but simply one or more basidia with the corresponding basidiospores. A teleutospore is *not set free from its place of origin* and therefore cannot be a factor in causing the dispersal of the fungus species in nature : it germinates *in situ*, whilst still attached to the tissues of its host.[1] A teleutospore is essentially a *resting structure*, *i.e.* a structure that can rest for a long period, especially through times of drought and cold, without injury ; so that, subsequently, when subjected to suitable conditions of temperature and moisture, it can revive and germinate. Many teleutospores, although able to rest without development for a long period, can, under favourable conditions, germinate at once, as in *Puccinia Malvacearum*. Other teleutospores, on the contrary, require a period of rest, generally the winter season, before they can germinate, as in *Puccinia graminis*. Usually teleutospores possess thick walls which contrast with the thin walls of the non-resting uredospores and basidiospores and which seem to be protective structures. Moreover teleutospores, at any rate in many Uredineae, can become quite independent of the cells from which they have arisen, except in the matter of attachment, and also independent of each other. Thus, for example, in *Puccinia graminis*, although in nature, in order to secure the successful dispersion of the basidiospores, the teleutospores which produce them must remain attached to the hyphae of the sorus on the host-plant, yet the teleutospores, if artificially torn from their natural positions, isolated, and placed under favourable conditions, will nevertheless germinate.[2]

[1] This rule applies to the great majority of the Uredineae, but is not quite of universal application, as there are a few exceptions to it. The exceptions will be discussed in the next Chapter.

[2] It must be admitted, however, that such germination is often abnormal, but this is owing to the difficulty of securing the right conditions of moisture in small closed chambers, etc.

The existence of the teleutospore in the Uredineae, to which there is nothing to correspond in the Hymenomycetes, is directly connected with the parasitic habit of the fungi concerned. The Uredineae are parasitic upon Phanerogams and Ferns, plants which have regular periods of vegetative development depending on the seasons of the year. The organisation of the Uredineae is perfectly adapted to fit in with this periodicity. And herein we find an explanation of the existence of the teleutospore : *the teleutospore is a cell-organ which provides for the rapid production of basidiospores when these can best be liberated with a chance of infecting new host-plants.* During summer, the mycelium which is to produce a teleutospore-sorus spends its energies in obtaining from the host tissues the substances which are eventually to be delivered to the basidiospores ; but these substances, instead of being sent straight forward into the basidia and thence into the basidiospores, are packed into the teleutospores, *i.e.* into probasidia or mother-cells of the basidia, there to be stored until a suitable time arrives for the production of the basidiospores. In *Puccinia Malvacearum*, this suitable time is the coming of damp weather when the air is saturated with moisture ; in *P. graminis* it is the spring when the second host, *Berberis vulgaris*, opens its winter buds and puts forth its tender green leaves.

Consider now the vernal condition of the haulms of an Oat plant bearing teleutospore-sori of *Puccinia graminis*. Neither the tissues of the host-plant nor the vegetative mycelium of the parasite are any longer living : only the teleutospores retain their vitality. Yet the teleutospores produce basidiospores successfully within a very few hours after the advent of moist weather. This is rendered possible by the teleutospores being organised as independent structures filled with all the food materials necessary to enable them to develop mature basidia. In being an independent resting body containing a store of reserve food-materials and in being destined to produce organs of dissemination [1] under favourable conditions, a teleutospore is analogous to a sclerotium.

[1] I apply the term *organs of dissemination* to seeds, spores of fungi, soredia of lichens, etc., *i.e.* to structures which are so organised that they are set free and by the agency of the wind, water, animals, etc., are transported from place to place, so that they disseminate the species.

The developmental season of the Uredineae is chiefly in the spring and summer and corresponds exactly to the developmental

Fig. 213.—Teleutospore-sori of *Puccinia Malvacearum* on the under side of a Hollyhock leaf (*Althaea rosea*). Photographed in England. Natural size.

period of the host-plants. On the other hand, most Hymenomycetes develop best in the autumn at a time when, as Linnaeus expressed it, "Flora is leading her Flowering Plants into winter quarters." This autumnal development is correlated with the fact that the

Hymenomycetes are mostly saprophytes and also with the fact that, every autumn, dead leaves, stems, and other structures which are discarded or broken away from Phanerogams form a new and rich substratum for saprophytic mycelia. Moreover, the damp autumn months constitute an admirable time for the infection of new substrata of this kind by the liberated spores.

We now come to the discussion of the form of the basidium in the Hymenomycetes and in the Uredineae ; but, for the present, we shall exclude from the former the Auricularieae and from the latter the Coleosporieae, for the Auricularieae and the Coleosporieae are exceptional in the nature of their basidia and will be treated of later. Let us compare the basidium which is typical for the Agaricineae, Polyporeae, etc., with that which is typical for Puccinia, etc. In the Hymenomycetes, the basidium is typically club-shaped : its central axis is straight ; and it bears terminally four sterigmata and four spores. In the Uredineae, the basidium is less compact ; it is usually somewhat elongated and bent at the end so that its whole form is sickle-shaped, its axis being curved ; and it bears four sterigmata and four spores laterally ; the position of the sterigmata is always on the convex side of the sickle. If we regard the interior of the mature basidia, we observe that, in the Hymenomycetes, the basidium is usually non-septate ; whilst, in the Uredineae, septa divide the basidium into four cells which lie in a linear series. The septate condition of the basidium and the lateral position of the spores in the basidium of the Uredineae has often been commented upon and contrasted with the non-septate condition of the basidium and the terminal position of the spores in the Hymenomycetes ; but, so far as I am aware, no one has remarked upon the fact that *in the Uredineae the basidium is typically curved whilst in the Hymenomycetes the basidium is typically straight.* The fact that the Uredineae have curved basidia has been frequently noticed, and various observers, from Tulasne and de Bary onwards, have published drawings showing incidentally that the basidia of numerous species of Uredineae are curved at their ends more or less semi-circularly ; but no one seems to have asked the question : what is the physiological meaning of the curvature ?

The question that we shall now attempt to answer is therefore :

what advantage does a Rust fungus gain by having its basidia curved ? In the Hymenomycetes, the basidia are closely packed together to form a hymenium, and they form a very compact layer with a comparatively smooth outer surface. Owing to the extreme crowding of the hymenial elements, it is necessary that the sterigmata should have a terminal position on each basidium. Moreover, since the sterigmata are always parallel to the basidium-axis and perpendicular to the surface of the hymenium, the spores which they produce are from the first directed away from the hymenium. The spores, when discharged, must therefore be shot perpendicularly outwards from the hymenium, *i.e.* in the best direction to give them a chance of escaping from the fruit-body. I consider that the form of the basidium in typical Hymenomycetes is perfect for securing efficiency in the production and liberation of spores from the hymenium. Its special features are: (1) its compact body, (2) its non-septate condition so that there is no expenditure of material in the production of interior cell-walls, (3) the short sterigmata in the construction of which but little material is used up, and (4) the delicate adjustment in the spatial relations of the sterigmata and spores. The sterigmata and spores are set as closely together as possible, having regard to the necessity of freedom for each spore during its development and discharge. If these four points are considered, it will be seen that the basidium in the Uredineae is not nearly so highly organised, for in the basidium of a Rust fungus we find : (1) that the basidium is often considerably elongated, (2) that three or four septa are always present which divide the end of the basidium into four cells, (3) that the sterigmata are often relatively long, and (4) that the sterigmata and spores are not closely and compactly arranged in relation to one another but, relatively, are sprawling. However, I am of the opinion that, although hymenomycetous basidia are admirably fitted for hymenomycetous fruit-bodies with their highly organised hymenia, yet uredinous basidia are just as admirably suited for their function in connection with uredinous fungi. In the Uredineae there is no hymenial layer such as we find in the Hymenomycetes. Instead, the basidia, on developing from the teleutospores, remain separated from each other. Now, in order that the sporidia shall be shot away from

the teleutospore-sorus with a good chance of escaping into the air so that they may be carried off by the wind, it is necessary that, immediately before their discharge, they shall be directed away from the sorus. The proper orientation of the sterigmata in space is accomplished in the Uredineae in the following manner. When the teleutospores germinate, the germ-tubes at first grow in a direction more or less parallel to the axis of the teleutospore and towards its exterior, *i.e.* more or less perpendicularly outwards from the surface of the teleutospore-sorus ; but, on emerging from the sorus, they soon begin to grow at their apices more rapidly on one side than on the other. In consequence of this differential lateral growth, which appears to be in no way dependent on external stimuli, the end of each basidium becomes curved. This curvature is usually arcuate in form and the extreme end of the basidium often becomes bent, with reference to the basidium-base, through an angle of 90 or more degrees. The convex side of the curved part of the basidium is directed away from the sorus and the concave side toward the sorus. A basidium, as soon as it has assumed its sickle-shaped form, ceases to grow in length and becomes divided by septa into four cells. Four sterigmata, one from each cell, are then developed *on the convex side* of the basidium and each sterigma soon gives rise to a terminal spore. Thus the sterigmata and the spores become more or less directed away from the sorus-surface (*cf.* Figs. 215, I and 216 ; pp. 535 and 537). In consequence of this arrangement, the spores when shot away from their sterigmata, stand an excellent chance of leaving the substratum on which the teleutospores have been developed, and of being caught and carried off by the wind. The explanation of the sickle-shaped form of the basidium in the Uredineae is now obvious : it is *a form which secures that the basidiospores shall be shot away from the teleutospore-sorus in a direction favourable to dispersion.* Were the basidia not curved, or were the sterigmata produced on the concave side of the basidium, it is obvious that basidiospore-discharge could not be anything like so successful as it actually is. In order to make clear the position of the basidiospores on the curved basidia in relation to the sorus as a whole, I have undertaken a special investigation upon *Puccinia Malvacearum.* The details of the investigation will

succeed this general discussion ; but the reader would do well at the present moment to consult Fig. 216 (p. 537) which illustrates a typical case of the orientation just described.

Summarising the above discussion, we may say that the Hymenomycetes and the Uredineae present us with two different methods for securing such an orientation of the basidiospores in space as will permit of their being successfully discharged. In the Hymenomycetes, the orientation consists of the following elements : (1) the basidium is straight and has its axis perpendicular to the plane of the hymenium ; (2) the sterigmata are terminal and have their axes parallel to the axis of the basidium and therefore perpendicular to the plane of the hymenium : and (3) each spore has a terminal position upon its sterigma. In the Uredineae, the orientation consists of the following elements : (1) the basidium at first grows with its axis more or less parallel to the axis of the teleutospore, but subsequently becomes curved ; (2) the sterigmata are developed on the convex outer side of the basidium : and (3) each spore has a terminal position on its sterigma. In consequence of these arrangements the sterigmata become directed in the Hymenomycetes away from the hymenium and in the Uredineae away from the teleutospore-sorus. The same end—successful discharge of the basidiospores—is thus provided for both by the straight basidium of the Hymenomycetes and by the curved basidium of the Uredineae.

It is to be noticed that the straight form of the basidium with the spores developed at one end is associated with the occurrence of a compact hymenium, whilst the curved basidium with the spores on the convex side of the curved end is associated with the isolation of the basidia and their production from a teleutospore. That the straight basidium, such as one sees in Agaricus, has a form best suited for a basidium that is an element in a compact hymenium seems obvious. In the Auricularieae where the basidia are septate, and in the Coleosporiaceae where the teleutospores function directly as basidia and become septate before the production of sterigmata, we have a distinct hymenium in which the basidia are held together by jelly. The basidia in both have straight axes : the sterigmata become more or less parallel to the basidium-axis and the position of the spores just before discharge is the same as in Agaricus,

i.e. at the outer end of the basidium : but here only one sterigma arises terminally whilst the other three arise laterally, one from each of the three lower cells. In order to convert the basidium of an Auricularia or of a Coleosporium into a basidium of the Agaricus type, all that would be necessary would be the removal of the septa and the sliding upwards of the place of origin of the sterigmata on to the top of the basidium. The essential arrangements of the basidium of an Agaricus, of an Auricularia, and of a Coleosporium for the shooting away of the spores, therefore, appear to be identical and stand in contrast to the essential arrangement of the basidium of a Puccinia. The basidium with a straight axis and with the mature spores occupying a position at the free end of the basidium is evidently the only suitable kind of basidium for a compact hymenium. On the other hand, the Puccinia type of basidium is excellently adapted for spore-discharge under conditions in which the formation of a compact hymenium is rendered impossible owing to the fact that the basidia must develop independently of one another from teleutospores packed irregularly.

Parasitism has been responsible for the origin of the teleutospore, the teleutospore for the isolation of the basidia and the non-production of a hymenium, and the isolation of the basidia for the curvature of the basidial shafts, curved shafts with sterigmata on the convex sides being for the discharge of the spores under the conditions provided for their development as efficient as, or possibly more efficient than, straight shafts with terminal sterigmata.

I regard the Agaricus type of basidium as having been evolved along with the formation of a compact hymenium, and do not think this type primitive. It is difficult to imagine how the Basidiomycetes were evolved and I shall therefore venture to speculate upon some of the steps in their phylogenetic development as follows. The first basidia of the primitive ancestral Basidiomycetes, probably, were produced on the mycelium as isolated structures, were transversely septate, and were provided with lateral sterigmata, so that at first each basidium was somewhat like the conidiophore of a Nectria, although, of course, from the nuclear point of view, profoundly different. We must assume that violent discharge of the basidiospores took place from the first. The most primitive

basidium probably soon became curved with the sterigmata and spores produced on the convex side of the filament, for this would favour the dissemination of the spores. We may suppose that the most primitive Basidiomycetes were saprophytes. Some of these fungi with curved basidia may have become parasitic, have then developed resting probasidia or teleutospores, and thus have become the Uredineae, whilst the others remained as saprophytes. Even at the present day there are certain hymenomycetous saprophytes with curved basidia, *e.g.* Helicobasidium. We may suppose that during the further evolution of these saprophytes the basidia became associated together to form a hymenium. With the development of a definite hymenium in which the basidia gave each other mutual support, the originally curved basidia may have become straightened with the retention of the transverse septation and the lateral production of sterigmata. This condition we find in the Auricularieae at the present day. The straightening of the basidium, however, may have taken place more than once—in the Auricularieae with the production of a gelatinous matrix and in the non-gelatinous Hymenomycetes without the production of such a matrix. The absence of a gelatinous matrix in the latter fungi, by bringing the basidia closer to one another, may have favoured the evolution of the hymenium from a somewhat loose to a perfectly compact form. As the compact hymenium underwent evolution in the non-gelatinous Hymenomycetes, the basidia probably lost their septa and changed from the cylindrical to the clavate form, while the sterigmata moved upwards to the top of the basidia, became reduced in size, and came to develop their spores so that the hila were all turned toward the basidium-axis.

Whilst making the investigations on the basidia of *Puccinia graminis* and *Endophyllum Euphorbiae-sylvaticae* which have already been described, I concentrated my attention chiefly on the phenomena connected with the discharge of the spores from the sterigmata, such as the excretion of the water-drop and the distance of spore-discharge, and it was only subsequently that I realised the importance of the curvature of the basidium. The drawings of the basidia of these fungi were therefore not made with any effort to lay stress on the curvature ; moreover, for the Puccinia,

the germination of the teleutospores was not shown in connection with the host-plant. I have therefore supplemented my researches on the two species in question by an investigation on *Puccinia Malvacearum* in which I have sought to make clear the relations of the basidia to the teleutospore-sorus upon the host-plant. A detailed account of these investigations will now be given which will serve to illustrate in a particular case the principles of organisation for the production and liberation of the basidiospores of Uredineae in general.

The Shape and Position of the Basidia of Puccinia Malvacearum during Spore-discharge.—*Puccinia Malvacearum* is one of the best-known of the Uredineae, largely because of its extremely frequent occurrence on the Hollyhock (*Althaea rosea*) grown in gardens. Taubenhaus[1] has contributed interesting facts concerning its morphology and life-history, while Robinson[2] has written a memoir on the relations of the fungus to the tissues of its host.

The material for my observations was obtained at first from an infected leaf of *Malva sylvestris* and subsequently from infected leaves of the Hollyhock. Part of a leaf of a Hollyhock showing numerous teleutospore-sori is reproduced from a photograph in Fig. 213 (p. 526).

A vertical section through a teleutospore-sorus is shown in Fig. 216 (p. 537). Owing to the action of the fungus, the leaf above an infected area undergoes hypertrophy so that it becomes depressed. At the same time the teleutospore-sorus, which is usually developed on the under side of the leaf, comes to bulge hemispherically downwards and thus gains an admirable shape for producing the maximum number of teleutospores in a position for the successful discharge of the basidiospores.

The distance to which the basidiospores are shot was determined in the following manner. A complete sorus of teleutospores was removed by a section cut through it in the plane of the lower

[1] J. J. Taubenhaus, "A contribution to our knowledge of the morphology and life history of *Puccinia Malvacearum* Mont.," *Phytopathology*, vol. i, 1911, pp. 55–62.

[2] W. Robinson, "On some relations between *Puccinia Malvacearum* Mont. and the tissues of its host plant (*Althaea rosea*)," *Mem. and Proc. of the Manchester Lit. and Phil. Soc.*, vol. 57, 1912–1913, pp. 1–24.

epidermis of the leaf. The hemispherical sorus was then turned upside down and set on a thin layer of 10 per cent. gelatine spread over the bottom of a compressor cell. The lid of the cell was then

Fig. 214.—*Puccinia Malvacearum.* Distance of discharge of the basidiospores. In the centre is a hemispherical sorus of teleutospores, cut from a leaf of a Hollyhock (*Althaea rosea*) and left resting on a layer of gelatine in a compressor cell for 16 hours. The teleutospores germinated and the basidiospores were shot away from the basidia in all directions. Drawn with a *camera lucida*. The distance to which the spores have been shot may be determined by reference to the scale. Magnification, 39.

set in place, so that the sorus became enclosed in a small glass chamber kept sufficiently moist by the gelatine. The cell was kept during one night at room temperature. Next morning, it was found that many of the teleutospores had germinated and that the basidia produced by them had discharged their spores in large numbers.

A *camera-lucida* drawing showing the distance to which the basidiospores were shot from the sorus in the still air of the compressor cell is shown in Fig. 214. An inspection of the drawing reveals that a large number of spores were shot a distance of 0·5 mm. and some 0·6–0·75 mm., and that the maximum distance of discharge (spore on the extreme right) was 0·85 mm. If the spore which had travelled 0·85 mm. away from the extreme right edge of the sorus was discharged not from the edge of the sorus but say from 0·15 mm. to the left of the edge, its total distance of horizontal projection must have been 1 mm.

Whilst Fig. 214 was being drawn, a number of basidia were still discharging spores. The excretion of a drop of water from the hilum of one of the spores in the course of a few seconds just before the spore was discharged was clearly perceived, although the magnification of the microscope was only 60.

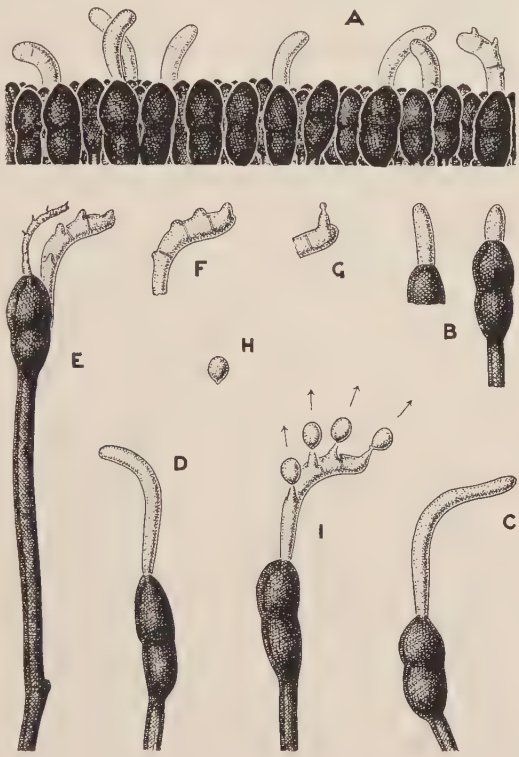


Fig. 215.—*Puccinia Malvacearum*. Development of curved basidia. A, section through a teleutospore-sorus from a Hollyhock leaf, a few hours after being placed in a damp compressor cell. Some of the teleutospores have germinated and have produced curved basidia. The one on the extreme right is developing sterigmata. B, spores with young basidia isolated from A: the basidia are at first straight and become curved only by further growth. C–H, elements isolated from a teleutospore-sorus on a leaf of *Malva sylvestris*. C, D, teleutospores with young curved basidia; E, a teleutospore with one collapsed basidium which has discharged its spores and one basidium about to develop spores; F, a basidium beginning to develop sterimata; G, a sterigma beginning to develop its spore; H, a discharged spore. I, semi-diagrammatic representation of a teleutospore which has produced a curved basidium with the sterigmata and spores on the convex side. The arrows indicate the probable paths of the basidiospores after discharge. Magnification, 293.

This observation goes to show that the mode of spore-discharge in *Puccinia Malvacearum* is exactly the same as in *Puccinia graminis*, *Endophyllum Euphorbiae-sylvaticae*, and the Uredineae in general.

The basidia which develop freely in air are curved like a sickle. Evidence of this was obtained in various ways, among others by isolating elements from a sorus of teleutospores upon a leaf which had been kept for a night in damp air. The drawings in Fig. 215, C, D, E, and F show such isolated elements; and I, which is a semi-diagrammatic drawing, shows a basidium with its four spores. The curvature of the elements is in all cases obvious, and it is to be noted that the sterigmata and spores are always developed on the convex side of the basidium so that they are directed away from the base of the teleutospore.

An attempt to watch the development of the basidia was made as follows. A vertical section through a piece of leaf including about one-half of a sorus was placed in a closed compressor cell on gelatine. Some of the teleutospores germinated (Fig. 215, A). At their first origin they were straight (B), but they soon became curved. Unfortunately the development of the basidia went no further than is shown in Fig. 215, A; but sufficient was seen to indicate that the basidia are always curved and that the sterigmata develop on the convex side.

The relations of the basidia to the teleutospore-sorus and to the leaf of the host-plant are shown in detail in Fig. 216, which represents a sorus in which a number of teleutospores have just germinated. Only some of the teleutospores germinate at any one time. After some have germinated, others germinate. Perhaps this is due to the fact that the teleutospores are not all developed simultaneously. The older ones are pushed on long pedicels toward the outside and younger ones are contained further within the sorus. Germination takes place only in moist-weather periods, especially after rains; and, during continued dry weather, no germination takes place at all. This I was able to observe by making daily observations on infected Hollyhocks growing in my father's garden at Birmingham, England. In Fig. 216, *a* shows very young basidia and *b* curved basidia with four full-grown spores. It will be noticed that all the full-grown basidia are curved more or less

FIG. 216.—*Puccinia Malvacearum*. A sorus of germinating teleutospores on the under side of a leaf of a Hollyhock (*Althaea rosea*). Owing to a depression in the leaf, caused by the fungus, the sorus bulges downwards convexly. Some of the teleutospores are producing basidia which are in various stages of development: *a a*, very young basidia; *b b*, basidia which have produced sterigmata and full-grown spores; *c*, a basidium shooting away its spores, one of which has excreted a drop of water from its hilum preparatory to discharge, and another of which has just been discharged carrying its drop with it; *d*, a basidium which has discharged three of its spores, one of which is seen carrying away its drop through the air; *e*, a basidium which has discharged all its spores, one of which is shown travelling away through the air and carrying its drop with it; *f f*, basidia which have collapsed subsequently to spore-discharge; *g*, a basidium in which the trajectories of two spores which are to be projected forward 0·3 mm., are indicated; *h*, a trajectory is shown with the distance of projection equal to 0·28 mm. Parts of other trajectories are shown at *c*, *d*, *e*, and *i*. The full average distance of violent projection, *i.e.* about 0·6 mm. (*cf.* Fig. 214, p. 534) or about twice the distance shown for the two spores at *g* and one spore at *h*, could not be represented in this Figure owing to lack of space. All the basidia are bent through about 90° and all bear sterigmata and spores on their convex side, so that the spores are shot more or less radially away from the centre of the sorus and thus have an excellent chance to escape into the air and be carried off by the wind. Magnification, 163.

through a right angle. At *c* is a basidium discharging its first spore which is carrying a drop of water with it through the air, while another spore has a drop of water of maximum size at its base and is about to be discharged. At *d* is a basidium which has discharged all its spores save one: the spore being projected forwards through the air is carrying its drop of water. From *e* all the spores have been discharged while at *f f* basidia are shown which have collapsed shortly after spore-discharge. At *g* and *h*, the trajectories of three spores in still air are shown by arrows. The distance due to the force of propulsion is only represented as being 0·3 mm. in *g* and 0·28 mm. in *h*, owing to want of space; but, as we have seen, spores are projected farther than this, often twice the distance shown at *g* and *h* (*cf.* Fig. 214, p. 534). By glancing around the Figure at *d*, *i*, *c*, *h*, *e*, and *g*, it will be seen that the spores, owing to their development on the convex side of the curved basidia, are discharged more or less radially outwards from the sorus centre. The advantage of the curvature of the basidium and of the spores being developed on the convex side will now be obvious. There is no doubt that the shape of the basidium favours effective spore-dispersal. If the spores were developed on the concave sides of the basidia instead of the convex, very few of them could escape.

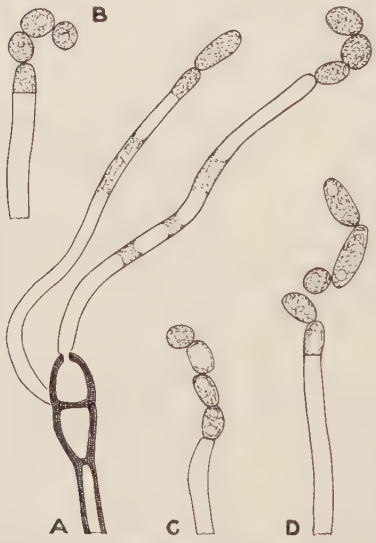


FIG. 217.—*Puccinia Malvacearum*. Development of basidia in a film of water. A section of a teleutospore-sorus from a Hollyhock leaf was placed in a compressor cell. The basidia here shown grew outwards in a film of water on the cover-glass. A, a teleutospore with two basidia: one of these has produced three end-conidia and the other, one. B, a basidium with three end-conidia; C, one with four; and D, one with four and a possible fifth. Magnification, 293.

It was found that some of the teleutospores in a compressor cell were germinating in a film of water condensed on the cover-glass. Under these conditions the basidia became unusually long and their ends became broken up into what Eriksson has called

end-conidia (Fig. 217). Usually these conidia were four in number (C and D), but sometimes they were three (A and B) and sometimes only one (A). These end-conidia are not violently discharged so that it is not astonishing to find that the curvature of the basidium, such as exists in basidia which are developed in air and which violently expel their spores, is not typically formed.

CHAPTER III

SPORE WALLS AND SPORE DISPERSAL

The Cell-walls of Basidiospores, Teleutospores, Uredospores, and Aecidiospores—Compact and Loose Teleutospore-sori—Spore-wall Pores—The Number of Uredospores and Aecidiospores of *Puccinia graminis*—The Violent Discharge of Aecidiospores—The Dispersal of the Spores by the Wind.

The Cell-walls of Basidiospores, Teleutospores, Uredospores, and Aecidiospores.—The long curved isolated basidia of the Uredineae are particularly dependent for their successful development upon the amount of moisture in the atmosphere: and it is not surprising, therefore, that they are only produced in moist weather and that, when moist weather comes, they develop and discharge their spores as quickly as possible. Now the walls of the basidiospores of the Uredineae, like those of the Tremellineae and of many non-gelatinous xylophilous Agaricineae, are always smooth and thin and colourless. These characteristics, here as in the other fungi, are correlated with the rapidity of development of the individual spores. A spore, if it is to be developed and discharged in a minimum amount of time, must have a very simple wall, *i.e.* the wall must be smooth and thin and colourless, for only such walls can be produced by the simplest means and by the least amount of physiological action.[1]

The walls of teleutospores are usually thick and impregnated with a brown pigment. Since teleutospores, relatively to the basidiospores, develop very slowly, there is plenty of time for this cell-wall elaboration. Teleutospores are resting bodies and often persist upon their hosts for weeks or months before germinating. Their thick walls probably have a protective significance. The brown pigment in the cell-walls may possibly be a waste product

[1] *Cf.* these *Researches*, vol. ii, 1922, pp. 49, 161.

of metabolism, but it is also possible that it may protect the spores, in certain species at least, from injurious light action. The teleutospore-sori of *Puccinia graminis*, etc., are jet black in the mass and thus resemble the black sporangia of Pilobolus and the black spores of Coprinus and Panaeolus. Now all these black structures are often exposed to strong sunlight for many weeks or months before they undergo development. The sori of the *Puccinia* remain attached to upright grass-stems, etc., all through the autumn and winter. The sporangia of Pilobolus, after being shot away

FIG. 218.—*Cronartium ribicola*. *a*, verrucose aecidiospore of *Peridermium Strobi* from a White Pine; *b*, teleutospore-columns on leaf of Red Currant; *c*, distantly and sharply echinulate uredospores, from Red Currant; *d*, smooth top of a column of teleutospores, from Red Currant. From W. B. Grove's *British Rust Fungi*. *b*, reduced; *a*, *c*, and *d*, magnification, 600.

from a dung mass, stick to grass leaves, grass stems, etc., until horses or cattle come along and swallow them; and the spores of many Coprini and Panaeoli are only successful in finding their way into dung when they have settled on grass, etc., and have been eaten by herbivorous animals. It is possible, therefore, that, since the sori of *Puccinia graminis*, the sporangium of Pilobolus, and the spores of Coprinus and Panaeolus must all, of necessity, be exposed to sunlight for many weeks or months, the deep brown or black pigment which is present in them may have a protective significance so far as certain rays of light are concerned. This, however, is a suggestion, the value of which can only be decided by direct experiment.

The uredospores and aecidiospores are usually sculptured upon

their exterior, the uredospores being echinulate or verrucose and the aecidiospores verrucose (Fig. 218, *a* and *c*). In contrast with the uredospores and aecidiospores, the teleutospores, with few exceptions, are smooth (Figs. 218, *d*, and 222, *b*, p. 546). This structural difference is correlated with a difference in function. The uredospores and aecidiospores act as organs of dissemination and, as such, are freely liberated and blown about as powder by the wind. Their rough exterior probably assists them in attaching themselves to the surface of their host-plants when they have once settled upon them. On the other hand, teleutospores, as a rule, are not organs of dissemination but probasidia destined merely to produce basidia upon which organs of dissemination (basidiospores) may arise. They are therefore anchored to their hosts, usually by stout pedicels, and are not set free. An echinulate surface would be of no use to them and is therefore not developed.

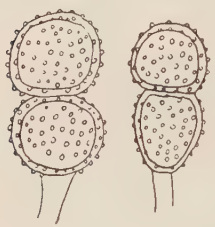


Fig. 219. — *Puccinia fusca*. Two teleutospores, very much constricted, composed of two almost globose or oblong cells which easily separate, walls densely covered with large warts. From W. B. Grove's *British Rust Fungi*. Magnification, 600.

As already indicated, there are a few Rust fungi which, in contrast with the vast majority, possess teleutospores which are not smooth but have sculptured walls. Several of these fungi, *e.g. Polythelis Pulsatillae*, *P. Thalictri*, and *P. fusca*, have pycnospores and teleutospores only, while the teleutospores are remarkable in being pulverulent (Fig. 219).[1] It appears therefore that, in the absence of uredospores and aecidiospores in these species, the teleutospores, while still retaining their probasidial function, have become detachable and thus converted into organs of dissemination. It seems probable that the walls of the teleutospores became sculptured as the teleutospore-sorus became pulverulent and some advantage could be gained by the teleutospores attaching themselves after transportation through the air, to their host-plants or other substrata upon which they happened to fall.

[1] J. C. Arthur, *North American Flora, The Uredineae*, vol. vii, part ii, 1907, pp. 152–153. Polythelis is one of the sub-divisions of the old genus Puccinia. Arthur states, for all three of the species I have mentioned, that the two cells of the teleutospore easily separate.

Compact and Loose Teleutospore-sori.—In many species of Puccinia, *e.g. Puccinia graminis*, the teleutospore-sorus is very compact : the teleutospores press against one another and thus form a continuous layer. The teleutospore is two-celled and, as if to make it easy for the lower cell of the two to send out its basidium, its germ-pore is usually situated as high up in its cell-wall as possible (Fig. 204, A, p. 505). In Phragmidium, *e.g. Phragmidium Rubi*, on the other hand, the teleutospore-sorus is very loose : the teleutospores do not press against one another but are isolated from one another (Fig. 220, *b* and *c*). This isolation of the individual teleutospores or, in other words, the provision of well-developed inter-teleutosporic spaces, is here of distinct advantage for the dispersal of the basidiospores. The teleutospores, instead of consisting of two cells only, as in Puccinia, consist of a row of cells, in *P. Rubi* 4 to 7 in number ; and each cell produces a basidium. The spatial separation of the teleutospores permits of each individual teleutospore producing a comparatively large number of basidia which are laterally situated but, nevertheless, provided with free space for the successful discharge of their basidiospores.

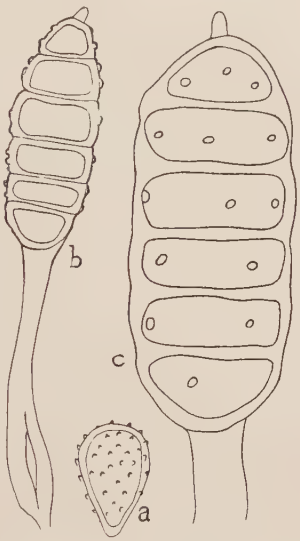


Fig. 220.—*Phragmidium Rubi*. *a*, a verrucose aecidiospore ; *b*, a teleutospore with a long pedicel and six cells ; *c*, a teleutospore, boiled in lactic acid for one minute, showing 2–3 germ-pores in each cell. On *Rubus fruticosus*. From W. B. Grove's *British Rust Fungi*. Magnification : *a*, 600 ; *b*, 360 ; *c*, 600.

Spore-wall Pores.—The walls of uredinous basidiospores are exceedingly thin, whilst those of teleutospores, uredospores, and, aecidiospores are relatively thick. Associated with this difference in wall thickness is the presence or absence of pores. The basidiospores, which have very thin walls, have no pores whatever and doubtless, like thin-walled hymenomycetous basidiospores, can send out a germ-tube anywhere at their surface. For a uredinous basidiospore under natural conditions the actual position in the wall for the

emergence of a germ-tube is probably decided by some stimulus coming from the epidermis of the host-plant or by the action of light.[1] Teleutospores, uredospores, and aecidiospores, which have relatively thick walls, are usually provided with one or more pores. Each spore produces a single germ-tube and the function of a pore is to provide a point of easy exit for a germ-tube. Where a spore has a single pore, the germ-tube emerges from that pore (Fig. 204, A, p. 505); and, where a spore has several pores, the germ-tube emerges from one of the pores.[2] When one or more pores are present in a spore, the germ-tube never emerges anywhere else than through a pore.

The teleutospore in Uromyces and Puccinia, *e.g. Uromyces Fabae*, *Puccinia graminis* (Fig. 204, A, p. 505), and *P. Phragmitis* (Fig. 222, *b*, p. 546) invariably has one pore for each cell; and this pore always defines the position of origin of a basidium. Most of the other genera, *e.g.* Gymnosporangium and Melampsora, have one pore or none for each cell. In many species of Phragmidium each cell has from two to three pores (Fig. 220, *c*). It is clear, therefore, that, in the Uredineae generally, if we except Phragmidium which has a loose teleutospore-sorus and many-celled teleutospores, the rule is that each cell of a teleutospore has one pore only when a pore is present at all.

The uredospore, in all the higher Uredineae, has from 2 to 10 pores, and in most species from 3 to 6. Thus the number of pores in each uredospore is: in *Puccinia Vincae*, 3 (Fig. 223, *c*, p. 547); in *Uromyces Fabae*, 3 to 4; in *Puccinia graminis* and *P. Phragmitis*, 4 (Fig. 222, *c*, p. 546); in *Hyalopsora Aspidiotus*, 4 to 8 (Fig. 221); and in *Puccinia dispersa*, 7 to 10.[3] Out of many hundreds of species included within the genera Uromyces and Puccinia there are only

[1] With the thin-walled poreless basidiospores of the Uredineae may be contrasted the thick-walled monoporous basidiospores of certain of the chromosporous Hymenomycetes, *e.g. Coprinus sterquilinus*. Here the pore is always terminal and from it the germ-tube always emerges.

[2] Occasionally where a uredospore has several pores, as with certain pollen grains having several pores, germ-tubes may emerge from two pores; but only one of the germ-tubes continues its development, while the other remains in a rudimentary condition.

[3] W. B. Grove, *The British Rust Fungi*, Cambridge, 1913, pp. 97, 177, 251, 260, 273, and 374.

two which have uniporous uredospores, namely, *Uromyces uniporulus* and *Puccinia monopora*.[1] The several pores of a single uredospore, *e.g.* of *Puccinia graminis* and *P. dispersa*, are always arranged on the walls at some distance apart.

The aecidiospore of most Uredineae, according to Sydow, Grove, and others, has numerous indistinct pores or often possibly none at all.[2]

In considering the significance of the position and number of the pores in teleutospores, uredospores, and aecidiospores, the function of the pores as germ-pores must be borne in mind. Since the pores of aecidiospores are so indistinct and since relatively little is known about them, we shall exclude aecidiospores from the following remarks and confine our attention to uredospores and teleutospores.

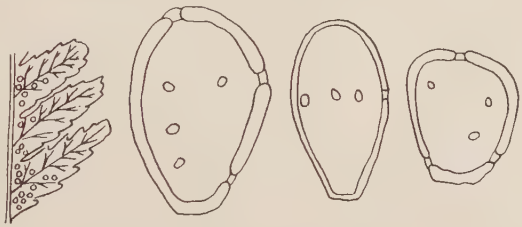


Fig. 221.—*Hyalopsora Aspidiotus.* To show pores in the walls of the uredospores. To the left, part of a frond of *Polypodium Dryopteris*, showing uredo-sori, natural size. To the right, three uredospores, all from the same sorus, the central one with 4 pores, the right-hand one with 6 pores, and the left-hand one with 7 pores, magnification 600. Material from Scotland. The faint, hardly perceptible warts on the exterior of the walls of the aecidiospores are not shown. From W. B. Grove's *British Rust Fungi*.

Why should a uredospore have several pores in its wall? I believe the answer is: to provide that at least one pore shall be on the side of the spore which happens to be turned towards the epidermis of the host-plant, and thus to assist the process of germination. Uredospores and pollen grains are analogous in the provision of, and functioning of, their pores. In the pollen grains of most Phanerogams, as in uredospores, there are several pores scattered upon the walls. Thus in *Colchicum autumnale* there are 2 pores; in most species, *e.g.* the Fire-weed, Willow-herb, Nettle, Oak, and Hazel, 3 pores; in the Alder and Birch 4 to 6 pores; in the Currant 8 to 12 pores; in the Convolvulus 15 to 18 pores; in the Carnation and Mezereon 20 to 30 pores; and in Nyctagineae over 30

[1] W. B. Grove, *loc. cit.*, p. 33.

[2] *Ibid.*, p. 31.

pores.[1] It is interesting to note the close similarity in appearance between certain pollen grains and uredospores. Thus the pollen grains of the Passion Flower, *Passiflora kermesina*, and the uredospores of *Puccinia Chondrillae* are almost exactly alike in the size and position of their three pores.[2] Pollen grains, before germinating successfully, must be deposited upon the surface of a stigma ; and uredospores, before germinating successfully, must be deposited upon the epidermis of some host-plant. Thus, both in pollen grains and in uredospores, germination must take place in such a way that a germ-tube shall penetrate the tissues upon which the pollen grains or uredospores happen to lie. The problem of germination under conditions of unilateral attachment has been solved in the same manner by both pollen grains and uredospores, *i.e.* several or many pores scattered in the cell-walls have been provided ; and this enables the germinating cells to send out their germ-tubes in the most favourable directions relatively to the tissues to be penetrated and thus to economise their food stores and growth energy.

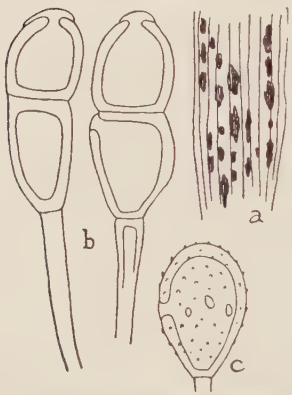


FIG. 222.—*Puccinia Phragmitis*. *a*, sori on leaf of *Phragmites communis* ; *b*, two teleutospores, with smooth walls, one germ-pore in each cell, and the germ-pore in the highest possible position in each cell ; *c*, a rough-coated uredospore with 4 equatorial germ-pores. From W. B. Grove's *British Rust Fungi*. *a*, natural size ; *b*, and *c*, magnification, 600.

In teleutospores the rule is, as we have seen, that each cell has one pore only. But teleutospores, with but few exceptions, are not organs of dissemination but *probasidia* which germinate in their place of origin and not upon the epidermis of a host-plant. Each cell of a teleutospore produces a basidium which grows outwards from the teleutospore-sorus ; and, to provide for the emission of the basidium, only one pore is necessary. The position of this pore is probably about the best possible, if the compactness of the teleutospore-sorus, the relative positions of adjacent teleutospores,

[1] Kerner and Oliver, *Natural History of Plants*, vol. ii, p. 98, Fig. 217.

[2] *Cf.* Kerner and Oliver, *loc. cit.*, Fig. 217, p. 98, and W. B. Grove, *loc. cit.*, Fig. 104, p. 152.

etc., be taken into account. Thus, in *Puccinia graminis* (Fig. 204, p. 505) and *P. Phragmitis* (Fig. 222), the upper cell of the teleutospore has a terminal pore and the lower cell a pore as near to the dividing wall as possible ; and it is obvious that these pore-positions facilitate the emergence of the germ-tubes into the outer air. The teleutospore and the uredospore of *P. graminis* and of other Uredineae have different problems to solve in their germination, and the difference in the number and position of their pores is an expression of their adaptation to carry out their special functions.

Among Pucciniae, other than *Puccinia graminis* and *P. Phragmitis*, having the pore of the lower cell of the teleutospore at the highest possible point are : *P. punctata* on Galium, *P. Buxi* on Buxus, *P. Malvacearum* on Malva, etc., *P. Calthae* on Caltha, and *P. Lychnidearum* on Lychnis.[1] But there are a number of exceptions to this rule, *e.g.* *Puccinia Vincae* on Vinca (Fig. 223, *a*, *b*), *P. Taraxaci* on Taraxacum, *P. major* on Crepis, *P. Primulae* on Primula, and *P. Pimpinellae* on Pimpinella.[2] Whether or not there is any biological advantage in the pores in these Pucciniae being situated low down in the cell-walls remains to be investigated.

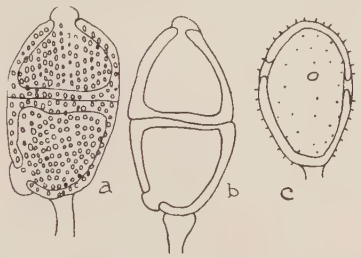


Fig. 223.—*Puccinia Vincae*. *a*, a teleutospore, seen dry, with the germ-pore of the lower cell near the pedicel ; *b*, the same, seen wet ; *c*, a uredospore, aculeate, having 3 germ-pores. From W. B. Grove's *British Rust Fungi*. Magnification, 600.

Why should the teleutospores of many species of Phragmidium differ from those of Uromyces and Puccinia in having three pores in each cell instead of one ? I am uncertain what is the correct answer to this question. The teleutospore of a Phragmidium is composed of a row of cells and each cell produces a short curved lateral basidium, so that several basidia may be produced by a single teleutospore at one time. It is possible that the cells of a teleutospore, when germinating, mutually influence one another

[1] For illustrations *vide* W. B. Grove, *The British Rust Fungi*, Cambridge, 1913, Figs. 115 (p. 165), 153 (p. 206), 154 (p. 207), 165 (p. 216), and 168 (p. 219).

[2] For illustrations *vide* W. B. Grove, *ibid.*, Figs. 106 (p. 154), 109 (p. 158), 127 (p. 177), 129 (p. 180), and 136 (p. 188).

in such a way as to cause the several basidia to arise in situations which will lead to least mutual basidial interference ; and to attain this end several exits for the basidium of each cell would be of advantage. However, exact observations only can decide the value of this suggestion and solve the problem which has been raised.

The Number of Uredospores and Aecidiospores of Puccinia graminis.—The number of aecidiospores, uredospores, teleutospores, and basidiospores produced by different species of Rust fungi varies greatly, more especially in accordance with the amount of infection of the host-plant. The following remarks will be confined to *Puccinia graminis.*

The number of uredospores of *Puccinia graminis* produced upon a single badly infected wheat-plant amounts to many millions ; and it is probable that the number of spores which come into existence upon the wheat of a single farm in Western Canada would often be far more than sufficient to infect all the wheat-plants in the world, could they be spread upon them and germinate under favourable conditions. When once uredospores have come to be produced in a wheat area, the extraordinarily rapid spread of the Black Stem Rust Disease in moist weather is exactly what one would expect to happen if due regard be given to the high geometrical increase in the number of the organs of dissemination in successive generations. In Volume I of this work, I described a puff-ball of *Lycoperdon giganteum* (*cf.* Fig. 224) which produced 7,000,000,000,000 spores. I have since calculated that, if every spore of this puff-ball had germinated and given rise to a puff-ball like its parent, and if every spore of the second-generation puff-balls had likewise germinated and given rise to a puff-ball like its parent, then, at the end of these two filial generations only, there would have come into existence a mass of puff-ball matter equal to 800 globes the size of the planet on which we live ! From a single uredospore which has infected the leaf or stem of a wheat-plant, several thousands of new uredospores may be produced in the course of seven or eight days, so that successive generations of uredospores can be produced under favourable conditions with great rapidity. On the other hand, in a Puff-ball, successive generations of spores are probably not produced in less than a year. After all, therefore, under ideal

conditions for propagation and in a given time, such a fungus as

Fig. 224.—*Lycoperdon giganteum.* A large white oval puff-ball, gathered August 4, 1915, from a field at Effingham, Surrey, England, just before the ripening of the spores. Weight, 5 lbs. 3 oz.; length, 13 inches; breadth, 11 inches; height, 9·5 inches; circumference, 3 feet 3·5 inches. Held, at the request of the author, by J. Ramsbottom. Photographed at the South Kensington Natural History Museum.

Puccinia graminis would achieve a greater increase in its numbers than even the Giant Puff-ball.

At Assiniboine Park, Winnipeg, in the middle of June, 1918, some three thousand Barberry shrubs (*Berberis vulgaris*) became very badly infected with *Puccinia graminis*, so that the leaves (Fig. 225) were discoloured with orange aecidiospore-pustules. In a letter to the *Manitoba Free Press*, I called attention to the danger to which the wheat-fields were exposed in consequence of the existence of these diseased shrubs. The result was that the Parks Board of the City of Winnipeg, acting with great promptitude, dug up all the shrubs within one week and burnt them. Before this destruction took place, however, I used the opportunity presented by the abundant fungus material to calculate the number of aecidiospores which the infected bushes were producing.

A single aecidium produced about 11,000 spores. This I found by counting the number of aecidiospore rows and the number of aecidiospores in each row in individual aecidia, and by then making a simple mathematical calculation. Now a single large pustule upon one of the Barberry leaves often contained 150 aecidia and a medium-sized pustule 50 aecidia (Figs. 225, 226). A large pustule therefore produced about 1,650,000 spores and a medium-sized pustule 550,000 spores. However, on each leaf, there were often several pustules. The number of aecidiospores produced on each leaf was therefore often several millions.

The number of spores produced on three well-infected living leaves was specially investigated.

The first leaf bore 17 small pustules. These, collectively, contained 336 aecidia, the number of aecidia in the 17 individual pustules being as follows: 35, 33, 32, 31, 31, 30, 28, 21, 16, 14, 13, 12, 11, 10, 8, 7, and 4. Reckoning 11,000 aecidiospores to each aecidium, the total number of aecidiospores produced by the leaf was 3,700,000.

The second leaf bore 7 large pustules and 3 smaller ones. These, collectively, contained 493 aecidia, the number of aecidia in the 10 individual pustules being as follows: 97, 74, 70, 63, 62, 47, 44, 29, 4, and 3. Reckoning 11,000 aecidiospores to each aecidium, the total number of aecidiospores produced by the leaf was 5,400,000.

The third leaf bore 3 very large pustules and 9 smaller ones. These, collectively, contained 732 aecidia, the number of aecidia

FIG. 225.—Leaves of the Common Barberry, *Berberis vulgaris*, obtained from shrubs in Assiniboine Park, Winnipeg, on June 17, 1918, bearing aecidia pustules of *Puccinia graminis*, the fungus which causes the Black Stem Rust Disease of Wheat. Each leaf was liberating several millions of aecidiospores. Shortly after the photograph was taken, as a result of a Barberry eradication campaign, more than 3,000 Barberry bushes in the Winnipeg parks were rooted up and burnt. Natural size.

in the 12 individual pustules being as follows : 178, 130, 127, 97, 74, 48, 28, 15, 11, 9, 8, and 7. Reckoning 11,000 aecidiospores to each aecidium, the total number of aecidiospores produced by the leaf was 8,000,000.

Fig. 226.—Four pustules of aecidia of *Puccinia graminis*, the Black Stem Rust Fungus, on the under side of a leaf of *Berberis vulgaris*, the Common Barberry. Each aecidium produced about 11,000 aecidiospores. Photographed by J. E. Howitt at the Ontario Agricultural College, Guelph. Magnified about three times.

From the above calculation it appears that a leaf of *Berberis vulgaris* well infected with *Puccinia graminis* produces from about 4,000,000 to 8,000,000 aecidiospores. A Barberry bush with 200 leaves, each leaf producing on the average 5,000,000 aecidiospores, would produce a total of 1,000,000,000 spores. It is probable that the 3,000 bushes infected with *Puccinia graminis* in the spring of 1918 at Winnipeg produced upwards of 2,000,000,000,000 aecidiospores.

The Violent Discharge of Aecidiospores.—In 1883, Zalewski[1] discovered that the aecidiospores of certain Uredineae, when ripe, are shot out of the aecidia with considerable violence. Since Zalewski's discovery seems to have been entirely overlooked by modern uredinologists and is nowhere mentioned in botanical text-books, I shall here describe it in detail.

Zalewski placed some leaves of *Euphorbia Cyparissias*, which bore aecidia of *Uromyces Pisi*, on glass slides under a bell-jar containing moist air in such a way that some of the aecidia looked directly upwards. He then suspended

[1] A. Zalewski, " Über Sporenabschnürung und Sporenabfallen bei den Pilzen," *Flora*, Jahrg. LXVI, 1883, pp. 268–270.

other slides, moistened on their under surfaces, at various distances above the aecidia. On the next day he found that the under sides of all the slides which had been suspended 10–15 mm. above the aecidia were densely covered with aecidiospores. On the under sides of slides suspended at greater and greater heights than 15 mm. the aecidiospores became fewer and fewer, until there were none at all. It was further observed that, where spore-discharge had not been hindered by suspended slides, the aecidiospores had settled on the leaf for some distance all round the outside of each aecidium. By the third day after the experiment had been set up the aecidia had freed themselves from spores almost down to the base of each cup.

When Zalewski tilted the Euphorbia leaves to an angle with the vertical, he found that the aecidiospores were discharged to a horizontal distance of 15–20 mm.

Zalewski successfully repeated the experiments made on the aecidia of *Uromyces Pisi* with aecidia of other species which he named as follows :

Puccinia straminis	on	*Lycopsis arvensis*,
Aecidium Symphyti	on	*Symphytum officinale*,
Puccinia Calystegiae	on	*Calystegia sepium*,
Puccinia coronata	on	Rhamnus leaves.

From the above series of experiments Zalewski came to the conclusion that, in the Uredineae, the aecidiospores of each aecidiospore-chain are violently and successively discharged from the aecidium, the oldest outermost spores being discharged first.

Zalewski also observed that there was greater violence of discharge from aecidia developed on thick leaves than on thin. Thus the aecidia on the thick leaves of the Euphorbia, the Lycopsis, and the Symphytum discharged their spores to a greater distance than the aecidia on the relatively thin leaves of the Calystegia (Convolvulus) and the Rhamnus. Whereas the aecidia on the Euphorbia leaves discharged their spores to a height of 10–15 mm. and even higher, the aecidia on the leaves of the Calystegia and the Rhamnus discharged their spores to a height of only 4–6 mm. and, at most, 8 mm. It was also observed that the aecidia of *Puccinia coronata*

discharged their spores to a greater height when on a much thickened petiole than when on a slightly thickened portion of a lamina.

Finally, Zalewski observed that the aecidiospores are shot out of their aecidia in dry air, although not so freely as in moist.

Dr. B. O. Dodge [1] of the Bureau of Plant Industry, Washington, has recently confirmed Zalewski's results, for he has observed violent aecidiospore-discharge in the following species :

Gymnoconia peckiana	on	*Rubus canadensis,*
Gymnosporangium myricatum	on	*Myrica cerifera,*
Puccinia Podophylli	on	*Podophyllum peltatum.*

The Podophyllum Rust shot its aecidiospores to a height of 1–1·5 cm. Dr. Dodge states that "the germ-pores of the aecidiospores of the Myrica and Podophyllum rusts are formed as a result of a thickening of the spore-wall : small plugs are formed which push into the spore opposite in such a way that, as the spore reaches maturity and is pushed upwards, it comes into a critical position where the resistance due to friction is not sufficient to balance the elastic forces, whereupon the spore is suddenly released and shot out to some distance. The spore-plug simply acts as an especially good fulcrum." And Dr. Dodge adds : "I think that without these plugs between the spores an aecidiospore would be shot out some distance when released. The orange rust, Gymnoconia, discharges its spores from the large caeomas with some force. There are no pore-plugs, at least none of any size, formed in connection with the germ-pores of this species."

In the spring of 1924, whilst in England, I myself confirmed Zalewski's results by observing violent aecidiospore-discharge in :

Uromyces Poae	on	*Ranunculus repens,*
Puccinia graminis	on	*Berberis vulgaris,*
Puccinia pulverulenta	on	*Epilobium hirsutum,*
Puccinia Poarum	on	*Tussilago Farfara.*[2]

[1] B. O. Dodge, *in litt.*

[2] Since this Chapter was set up in type, Mr. J. H. Craigie of the University of Minnesota has informed me *in litt.* that he has observed violent aecidiospore-discharge in : *Puccinia fraxinata, P. Clematidis, P. Impatientis, P. Grossulariae, P. urticata, P. hieraciata, P. coronata,* and *P. graminis.*

(1) *Uromyces Poae.* The first experiment was made by Mr. W. B. Grove and the author working in conjunction. The leaves of the Buttercup were laid out in a dish so that the aecidia looked upwards, and slides smeared with glycerine were set above them. The spores were shot upwards : for, a day or two after the beginning of the experiment, many of them were found stuck to the under side of the slides at a height of 2–3 mm. above the cluster-cups, while others were seen to have collected on the leaves in a zone around the cluster-cups. The maximum vertical distance of discharge observed was 9 mm. At the end of 3–4 days, the cups were empty almost to their base.

On June 7, I collected some Buttercup leaves bearing *Uromyces Poae* and, the next morning, placed a portion of a leaf flat in a compressor cell (*cf.* Fig. 103, p. 240), so that the aecidia looked upwards. A small drop of water was then added to the preparation in such a way that it did not touch the aecidia. After closing the cell and pressing the lid down to within about 1 mm. of the upper surface of the leaf, I perceived with the low power of the microscope that the cups were almost empty, most of the aecidiospores having been discharged. Nevertheless, spore-discharge was still in progress ; for, on focussing the cover-glass, I saw some of the remaining spores strike and stick to its under surface. In the course of 24 hours several hundred spores collected in this way, thus forming an orange spore-deposit. It became evident that the aecidiospores were being discharged one by one in succession, and not as clouds comparable with those produced during the puffing of Pezizae, and that the discharged spores were turgid.

(2) *Puccinia graminis.* Notwithstanding the large amount of attention which has been paid to *Puccinia graminis* as the cause of Black Stem Rust Disease of wheat and other cereals, hitherto, so far as I know, no one has ever observed the violent discharge of the aecidiospores.

On June 19, with the help of a pair of scissors, I cut out from a Common Barberry leaf a small piece of lamina upon which were a number of aecidia (*cf.* Figs. 225 and 226, pp. 551 and 552), and mounted the piece of leaf on a small drop of water in a compressor cell, so that the cut edges of the piece of leaf were wetted and the

aecidia looked upwards. The material had been gathered for a day or two before it came into my possession and had become somewhat dry. The piece of leaf in the compressor cell, therefore, soon began to absorb water through its edges. After about two hours from the closing of the compressor cell, the aecidiospores began to be shot out of their cups. I saw them strike and stick to the under surface of the cover-glass, just as in *Uromyces Poae*, and many hundreds of them were shot up to a height of 2–3 mm.

Further observations were made with the help not only of a compressor cell but also of a glass-ring cell which was 10 mm. high. During the night, in one compressor cell a number of spores were shot upwards to a height of 4 mm., while in the ring-cell a few isolated spores were shot up 7·5 mm. My observations seem to show that the maximum height of discharge for the individual aecidiospores of *Puccinia graminis* is 7–8 mm.

On focussing the top of the chains of aecidiospores in the cluster-cups, I saw some of the spores discharged. At one moment a spore could be seen at the end of its chain, at the next moment it suddenly disappeared : its actual flight could not be observed. Sometimes spores were shot away not individually but in groups of two, three, or more. Several times the aecidia discharged what we may call *aecidiospore-bombs*, *i.e.* clumps of spores some of which contained 60 to 150 individual spores. The sides of some aecidia seemed to blow out like the side of a long-dormant volcanic peak which eventually suffers from a violent eruption, for along with an ejected aecidiospore-bomb a larger or smaller fragment of the colourless peridium was often carried. Some of the aecidiospore-bombs landed on the under side of the cover-glass at a height of 7–8 mm. above the aecidia, while others struck the side of a large ring-cell at a distance of fully 1 cm. from the cup which had produced them, thus affording evidence that they had been shot outwards with considerable violence. In some of the ejected aecidiospore-bombs more or less isolated chains of aecidiospores could be clearly seen, from which fact we obtain some indication of the depth in the aecidial crater at which the explosive force had come into existence.

When an aecidiospore-pustule was placed in a ring-cell so that the tops of the aecidia were 7–8 mm. below the cover-glass, only a very

few individual spores and a few large bombs struck and adhered to the under side of the cover-glass, while the majority of the spores, after being shot up into the air at various angles, fell back again either into the aecidial craters or on to the epidermis of the leaf outside. Thus a distinct yellow halo of spores, 3–4 mm. wide, was gradually formed around each aecidiospore-pustule.

Upon a discharged aecidiospore, clinging to its outer wall, may usually be seen one or more tiny colourless droplets. These do not consist of water, for they do not rapidly evaporate, but their appearance suggests mucilage. Up to the present I have not had an opportunity to investigate them chemically, but I suspect that they owe their origin to the intercalary cells in the aecidiospore-chains and play a part in the phenomenon of discharge.

(3) *Puccinia pulverulenta.* As before, small pieces of Willow-herb leaf bearing aecidia were mounted on a tiny drop of water in a compressor cell. Within five minutes of closing the cell aecidiospores were seen striking the cover-glass. Many spores were shot upwards 2–3 mm., some 4 mm., but none 9 mm. A bomb of about 50 spores was shot up 4–5 mm. Tiny colourless droplets were seen clinging to the discharged spores, just as in *Puccinia graminis.*

(4) *Puccinia Poarum* (= *P. epiphylla*). A piece of a Coltsfoot leaf containing an aecidium-pustule was mounted on a drop of water in a ring-cell. Some of the aecidiospores were soon seen to strike the under side of a cover-glass at a height of 7 mm. above the mouths of the aecidia. Two ring-cells were placed one above the other and a cover-glass on the top. After setting the piece of leaf at various heights from the base of the compound cell, it was found that some of the spores were discharged to a height of 1·1 cm. but none to a height of 1·5 cm. When the outermost aecidiospores in an aecidium were kept in focus, the discharge of individual spores was observed. In its flight a spore often carried away one or more loose spores which lay in its path. Little colourless droplets were observed on all the discharged spores, as in *Puccinia graminis* and *P. pulverulenta.*

In concluding this Section, a few general remarks may be added. The curious structure of the aecidium with its peridium and its

numerous compact rows of closely fitting many-sided aecidiospores should hereafter be conceived of as correlated not merely with the mode of production of the spores but also with the mechanism for discharging them. In the past no one seems to have understood this sufficiently. Hence the silence in our text-books in respect to aecidial gunnery. There is every reason to believe that all typical aecidia discharge their spores violently.

The exact cause of aecidiospore-discharge is not fully clear to me. Discharge only takes place when the aecidiospores are sufficiently moist and therefore when the aecidiospores are turgid; and this suggests that the turgor of the ripe spores is an important factor in the process. Where, at the end of a chain, two aecidiospores are attached together, the common wall is flat. Here, it may well be, two opposing forces come into play: (1) the adhesion of the two spores to one another, due, as we may suppose, to the presence of a cementing intercalary-cell substance, and (2) the pressure of the cell-contents of the spores, this pressure tending to round off the spores and thus to make the walls cemented together convex instead of flat. When the spores are ripening, we may suppose that the adhesion of the two end spores to one another becomes lessened or that their turgidity increases, until, at a particular moment, the force of adhesion is overcome by the force due to the cell-contents, the result being the sudden and violent discharge of the terminal spore. However, each terminal spore in a spore-chain is in contact not only with a subjacent spore at its base, but with several other spores, belonging to other spore-chains, round its lower half. This fact suggests that the same pair of antagonistic forces which we have supposed comes into play between the terminal and subterminal spores in a chain also comes into play between a terminal spore and the spores by which it is surrounded. Thus it may well be that the discharge of each spore is due not merely to the overcoming of the force by which it adheres to the subterminal spore by the force due to the turgor of itself and the subterminal spore, but also due to the action of similar antagonistic forces between itself and the spores surrounding its lower half.

The significance of violent aecidiospore-discharge is doubtless

the same as that of violent basidiospore-discharge, *i.e.* violent discharge is a means of separating the moist adhesive spores from their moist and living substratum and ejecting them into the air in such a way that they may be carried off by the wind and thus aid in the dispersal of the species. The discharge of aecidiospores to a distance, varying with the species, of from 4 to 15 mm. is violent enough to eject even the last-formed spores at the bottom of the aecidia into the air well beyond the rim of the peridium. The opening out of the peridium, its radial splitting into rays, and its becoming more or less revolute make for efficiency in the liberation of the spores ; for, by functioning in this way, the peridium forms as small an obstacle as possible to the escape of the spores as they are shot outwards from the interior of the aecidium.

The peridium is composed of living cells which fit together to form a continuous sheet of cells one cell in thickness. In some species, *e.g. Puccinia Caricis* (aecidia on Nettle), on the outside of the peridium, the wall is much thicker than on the inside and a piece of the cell-wall of one cell stretches downwards and overlaps the upper part of the cell-wall of the next lower cell, so that between two successive peridial cells there is a kind of hinge. This disposition of the cell-walls is doubtless of functional significance. As the peridial cells increase in length in a direction parallel to that of the axis of the aecidium, their thicker outer walls stretch less than their thinner inner walls and, at the same time, the outer thicker wall of the peridium as a whole tends to preserve its continuity, while bending at its hinges, whereas its inner wall tends to lose its continuity owing to the cells rounding themselves off. Hence, as growth takes place, the peridium readily bursts, opens out, splits radially into rays, and becomes revolute. To what extent, if any, the lower part of the peridium, which encloses and presses against the tightly packed chains of spores contained within the aecidium, assists in providing the force employed in the discharge of the spores remains to be investigated experimentally.

The Dispersal of the Spores by the Wind. — A uredinous basidiospore which has been shot away from its sterigma into the air is carried off by the wind in the same manner as the spores of the Hymenomycetes. Owing to its small mass, its relatively

enormous surface area, and its strongly curved exterior, it must dry up, after liberation into air not completely saturated with moisture, within a few seconds.

The rate of fall of uredinous basidiospores in still air, which is of considerable interest in connection with a number of phytopathological problems, has not yet been directly measured; but, from our knowledge of the size of the spores and of the rates of fall of hymenomycetous basidiospores of different sizes as given in the first Volume of this work,[1] there can be but little doubt that it is only about 1–3 mm. per second, the exact speed depending upon the spore's state of desiccation. When the wind is blowing at the rate of a few miles an hour, there is no theoretical reason why, considering the frequent reflection of the wind from obstacles at the surface of the earth and the irregularity of convection currents, basidiospores should not often be carried many miles before settling. Let us suppose, for instance, (1) that some bushes of Ribes infected with *Cronartium ribicola* are situated in a valley on a hill rising 2,000 feet above a surrounding plain, (2) that the wind blowing at the rate of 10 miles an hour sweeps up the valley and carries a stream of basidiospores from the Ribes bushes over the top of the hill and across the plain, (3) that the basidiospores in still air fall at the rate of 3 mm. per second, and (4) that the wind current carrying the spores from the top of the hill progresses in a direction parallel to the plain over which it passes. Accepting these assumptions, it is clear that the basidiospores would need to fall by their own weight through 2,000 feet of air before reaching the earth. Now since the rate of fall is 3 mm. per second and the distance of fall is 2,000 feet which equals 600,000 mm., the time taken for the spores to descend to the earth whilst being borne from the top of the hill across the plain would be 200,000 seconds or 56 hours; and, since the wind blows at the rate of 10 miles per hour, the spores, during these 56 hours and before settling, would be borne away from the hill a distance of 560 miles. If the hill were only 1,000 feet high instead of 2,000 and the speed of the wind only 5 miles an hour instead of 10, the spores before settling would be carried 140 miles; while, if the hill were only 500 feet high and the

[1] These *Researches*, vol. i, 1909, p. 175.

speed of the wind only 1 mile per hour, the spores before settling would still travel 14 miles.[1]

In the above theoretical case, the assumptions made were of the simplest kind, so that the problem of the carriage of the spores could be easily solved; but, in nature, the infected Ribes bushes are often situated amid low-lying forests, and the currents of air blowing over the surface of land covered by vegetation take the most various courses owing to the unevenness of the land surface, the irregular disposition of trees, shrubs, and herbs, local convection currents, and the change of winds. Truly, unless one is prepared to make an almost infinite series of observations: "The wind bloweth where it listeth, and thou . . . canst not tell whence it cometh and whither it goeth." Moreover, if to the uncertainty of our knowledge concerning the direction and speed of air-currents in forests, etc., be added our even yet considerable ignorance of the conditions of spore germination and infection in nature, the distribution of the infected plants of the two hosts of a heteroecious rust-fungus, such as Ribes and *Pinus Strobus* for *Cronartium ribicola*, should be for the present—as it actually is—often not a little perplexing to field observers.

Uredospores differ from basidiospores and aecidiospores in that they are not violently shot away from the places where they are developed. They accumulate at the surface of uredospore-sori, and it is not until the wind is blowing with a certain speed that they are carried away. Thus their dispersal is initiated like that of the spores of Puff-balls and of Mycetozoa. Uredospores, of course, are carried about by air-currents in the same manner as basidiospores;

[1] According to Perley Spaulding ("Investigations of the White-Pine Blister Rust," U.S. Department of Agriculture, *Bull.* No. 957, 1922, pp. 67–68), the experiments of York, Overholts, and Taylor indicate that the basidiospores of *C. ribicola* are very short-lived and, after their liberation, can scarcely survive drying for a period of 10 minutes. Abundant moisture is evidently necessary for infection of pines. It seems not unlikely that in nature, under moist conditions, the basidiospores, after being shot from their sterigmata, retain their vitality and power of germination for a much longer time than 10 minutes. However, the problem discussed above is one of the distance to which the basidiospores may be carried by the wind, not one of spore-germination. It is possible that the basidiospores, when carried long distances by the wind, cannot infect pines upon which they settle owing to loss of vitality in transit.

but, owing to their larger size, they fall in still air somewhat faster than basidiospores, and are transported by the wind proportionately shorter distances.

In Coleosporium the uredospores are produced in basipetal chains below an epidermis the top of which finally breaks open in an irregular manner and is reminiscent of a peridium. It seemed of interest to determine whether or not these spores, whose mode of origin superficially resembles that of aecidospores, are shot out from their sori like aecidiospores. I therefore examined *Coleosporium Tussilaginis* and *C. Petasitis* from this point of view, placing pieces of the leaves bearing the uredospore-sori in a compressor cell in the manner already described for aecidia in the previous Section. It soon became apparent that the uredospores were not being shot away at all but were lying as a loose powder within the peridium. There can be no doubt that the dispersal of these uredospores, like that of uredospores in general, is effected merely by the wind which carries them away from their place of origin.

An interesting example of the dispersal of aecidiospores is shown in the accompanying photograph reproduced in Fig. 227. In the centre is a young White Pine, *Pinus Strobus*, bearing numerous aecidia of the White Pine Blister Rust, *Cronartium ribicola*. These, known as *Peridermium Strobi*, were full of powdery aecidiospores. The tree was struck smartly with a stick, and immediately thereafter Dr. Güssow photographed the escaping spore-cloud which is to be seen on the left side of the tree being carried off by the wind.[1]

During high winds, gales, cyclones, etc., enormous numbers of spores of many species are carried up into the air often, doubtless, to considerable heights. It is easily conceivable that spores which have been raised in this way should be borne horizontally for scores or hundreds of miles before they settle. I am inclined to think that long-distance transportation of this kind accounts for the almost simultaneous infection of vast tracts of wheat in western Canada by *Puccinia graminis*. It may be that countless millions

[1] Whether or not the aecidiospores contained in the kind of peridium known as *peridermium*, such as that of *Cronartium ribicola*, are violently discharged like those of a typical aecidium, such as that of *Puccinia graminis*, is at present unknown.

of uredospores are picked up by the winds in some region in the United States where *Puccinia graminis* is flourishing, that they are dropped as a fine powder over many square miles of wheat-growing territory in Manitoba and Saskatchewan, that thereby the growing

FIG. 227.—Dispersal of aecidiospores. The young White Pine, *Pinus Strobus*, in the fore-ground bears numerous aecidia of the White Pine Blister Rust, *Cronartium ribicola*. The tree was struck smartly with a stick, about 3 feet above the blisters. As a result vast numbers of aecidiospores were set free. The spore-cloud thus formed can be seen to the left of the tree. Photographed at Wanensburg, New York State, by H. T. Güssow.

wheat-plants come to have uredospores lodged upon their stems and leaves, and that subsequently, with the advent of very moist weather, these spores germinate and infect large numbers of plants simultaneously. A new crop of uredospores would then be provided which, in their turn, might be caught up by the wind and infect new wheat-growing areas elsewhere.

The enormous transporting power of high winds, so far as fine dust is concerned, is easy to establish theoretically, although we still know but little about it from direct observations. During

gales in spring and autumn in Manitoba I have sometimes noticed that the air becomes distinctly hazy from the vast amount of extremely fine dust which has been picked up from the ground. Under such conditions the organic constitutents of the suspended dust are considerable. In the course of a series of experiments carried out every week throughout a whole year to determine the number of bacteria in the air of Winnipeg, C. W. Lowe and the author [1] found that during high gales as many as 8,000 bacteria were falling on each square foot of ground per minute. Now since these gales persisted for about 24 hours each, the air being dust-laden all the time, it is clear that after each gale, as the dust in the air settles, every square foot of earth must receive a vast number of new bacteria which have been transported by the wind for a considerable distance. Unfortunately, we have but few data concerning the dispersion of uredospores. However, the number of uredospores which are caught up by high winds from certain wheat fields is doubtless inconceivable : and, if we could only follow these uredospores and map out their places of lodgment, we should probably sometimes find them dispersed over hundreds of square miles stretching outwards in the direction of the prevailing wind and away from the source of the spores. The uredospores would settle down after a gale like the bacteria already spoken of, although doubtless in much smaller numbers.

In support of the theory of long-distance wind-dispersal as a factor in outbreaks of the Black Stem Rust Disease of wheat, may be cited the observations of Stakman, Henry, Curran, and Christopher, made in the United States with the help of aeroplanes.[2] In the spring and summer of 1921, spore-traps (vaselined glass slides) were exposed on aeroplanes in the Mississippi Valley at various places and at various altitudes ; and many spores of pathogenic fungi, as well as conidiophores, pollen grains, glumes of grasses, and small insects were caught. Spores and pollen grains were

[1] A. H. R. Buller and C. W. Lowe, "Upon the Number of Micro-organisms in the Air of Winnipeg," *Trans. Roy. Soc. of Canada*, Third series, vol. iv, section iv, 1911, pp. 51, 53, 55 ; also Plates I and II.

[2] E. C. Stakman, A. W. Henry, G. C. Curran and W. N. Christopher, "Spores in the Upper Air," *Journal of Agricultural Research*, vol. xxiv, 1923, pp. 599–606, Plates I and II.

relatively numerous up to 11,000 feet, but were much scarcer at greater heights. Two spores of what appeared to be *Puccinia triticina* (the cause of the Leaf Rust Disease of wheat) were caught at a height of 16,500 feet or, roughly, 3 miles above the ground. Uredospores and aecidiospores of *Puccinia graminis* caught at elevations of 7,000 feet and 1,000 feet, respectively, were found able to germinate.

Further evidence of long-distance transportation by the wind is provided by the observations of L. H. Pennington [1] on the spread of *Cronartium ribicola* from pines to currant bushes. Recently Pennington observed that, in the Pacific North-West of North America (which region has lately been invaded from the east by *C. ribicola*), certain currant bushes have become infected by the fungus and bear uredo-sori, although they are situated 110 miles beyond the limit of the geographical distribution of the White Pine (*Pinus Strobus*) from which the infecting aecidiospores must have come.

Not only spores of fungi but any other microscopic particles must, on account of their slow terminal rate of fall in still air, often be carried long distances by the wind before they come to earth. This has been shown to be the case for Lycopodium powder and pollen grains by Schmidt and Hesselman. Schmidt [2] shows that "Lycopodium spores which have a rate of fall of 1·76 cm. a second, when liberated in a wind blowing 36 kilometres (22·5 miles) an hour possess an average limit of distribution of 330 kilometres (206·25 miles). Similarly, even in the case of *Pinus sylvestris* pollen, which has as great a diameter as 48 μ, and as rapid a fall as 5·3 cm. a second, he computes that as many as four-tenths of the number originally shed would be carried 4 kilometres by a wind of 36 kilometres an hour; while two-tenths would reach 13 kilometres; and one-hundredth would reach 36 kilometres. The work of Hesselman [3] shows that these calculations represent the conditions attained in nature. He found that conifer pollen grains 30 to 60 μ

[1] L. H. Pennington, "Wind Dissemination of Aeciospores of Cronartium ribicola," *Phytopathology*, vol. xiv, 1924, pp. 52–53.

[2] W. Schmidt, "Die Verbreitung von Samen und Blütenstaub durch Luftbewegung," *Österr. Bot. Zeitschrift*, Jahrg. LXVII, 1918, pp. 313–328.

[3] H. Hesselman, "Iakttagelser över skogsträdspollens spridninsförmaga (Beobachtungen über die Verbreitungsfähigkeit des Waldbaumpollens)." In *Meddel. Stratens Skogsförsöksanst* (Sweden), Häft XVI, 1919, pp. 27–60.

in diameter were deposited in relatively vast numbers in dishes on lightships 30 kilometres (18·6 miles) and 55 kilometres (34 miles) from land; while pollen of grasses also, in spite of being liberated in far less abundance, nearer the earth, and unequipped with appendages for buoyancy, was caught in considerable quantities."[1]

McCubbin[2] has measured approximately the rate of fall of the uredospores of *Cronartium ribicola*, the White Pine Blister-Rust Fungus, in still air. He released air-dried uredospores instantaneously and automatically at the upper end of a long cardboard tube 9 inches in diameter, and allowed them to fall down the tube a distance of 8 feet on to glass slides filmed with glycerine. The slides were set on a flat disk which revolved slowly and uniformly just below the base of the tube. A small projecting pin on the revolving disk tripped the release apparatus at the beginning of a revolution and, as the slides passed successively under the tube, they caught the falling spores. The glycerine film was then scraped carefully into a line in the centre of the slide, the spores counted, and their number recorded in graph form. Some of the spores took five minutes to fall the 8 feet, *i.e.* they fell with a steady terminal velocity of 8 mm. per second. McCubbin remarks: "If such spores take approximately five minutes to fall a distance of 8 feet, one may get some idea of their transport distance by noting that in five minutes a 30-mile breeze would have carried them 2·5 miles. In other words, if these spores were set free 8 feet above the surface of a level plain, a 30-mile breeze would carry them 2·5 miles before they could sink to earth or, if liberated from a small hill 32 feet above the plain, they would be carried 10 miles by the same breeze before being deposited." McCubbin rightly concludes from his experiments that under windy weather-conditions "the radius of dispersal of the urediniospores of *Cronartium ribicola* is to be considered in terms of miles rather than of yards." Doubtless this conclusion applies to the uredospores of the Uredineae in general.

[1] W. H. Weston, Jr., "Production and Dispersal of Conidia in the Philippine Sclerosporas of Maise," *Journ. of Agric. Research*, vol. xxiii, 1923, pp. 263–264. The papers of Schmidt and Hesselman, at the time of writing, are inaccessible to me.

[2] W. A. McCubbin, "Dispersal distance of urediniospores of *Cronartium ribicola* as indicated by their rate of fall in still air," *Phytopathology*, vol. viii, 1918, pp. 35–36.

GENERAL SUMMARY

THE FOLLOWING IS A SUMMARY OF THE MORE IMPORTANT RESULTS OBTAINED DURING THE INVESTIGATIONS

PART I

Chapter I.—In the Psathyrella Sub-type the fruit-body is ephemeral: it opens, sheds its spores, and collapses within a period of about 24 hours. The gills are not mottled. The basidia are tetramorphic, *i.e.* are of four different lengths, and represent four distinct basidial generations with overlapping periods of spore-development. The paraphyses are large and form a hymenial pavement in which the basidia are set. The tetramorphism of the basidia permits of the basidia being laterally crowded, the spores of the longer basidia overstanding those of the shorter ones; and the lateral crowding of the basidia leads to an increase in the number of spores produced per unit area of hymenial surface. The small number of basidial generations and their overlapping periods of development are correlated with the light structure and the ephemeral existence of the fruit-body as a whole.

The fruit-body of *Lepiota cepaestipes*, which belongs to the Psathyrella Sub-type of organisation, is described and illustrated. The spore-discharge period was found to be of only about 15 hours duration. The longest basidia discharge their spores first, the next longest next, and so on for the two succeeding basidial generations until all the spores have been liberated.

Lepiota procera does not belong to the Psathyrella Sub-type. Its fruit-bodies are not lightly built and ephemeral but stoutly built and relatively persistent. The length of the spore-discharge period, under natural conditions, was found to be 8 days. The basidia are not tetramorphic but monomorphic. The general organisation of the hymenium appears to be very similar to that of *Panaeolus campanulatus*.

Chapter II.—*Psathyrella disseminata* belongs to the Psathyrella Sub-type. Although resembling *Lepiota cepaestipes* in its general mode of producing and liberating its spores, *P. disseminata* is not closely related to that fungus genetically.

Psathyrella disseminata produces a red ozonium, not hitherto de-

scribed, which grows out from bark or wood through the soil. On this ozonium the fruit-bodies are eventually developed. The existence of the ozonium accounts for the fact that thousands of fruit-bodies are often seen coming up simultaneously on bare soil surrounding the stump of a tree or covering dead roots.

An ozonium has two functions: (1) it serves to spread the fungus locally in a rapid manner, and (2) it enables the fruit-bodies to arise in more suitable locations than would often be possible, were these organs always obliged to arise directly upon a non-specialised mycelium at the surface of the nutrient substratum (wood, bark, etc.). The well-known ozonium of *Coprinus domesticus* functions in a similar manner to that of *Psathyrella disseminata*. The occasional occurrence of fruit-bodies of *P. disseminata* and of *Merulius lacrymans* on the surface of brick walls is illustrated and explained.

The author describes the structure of a fruit-body of *Psathyrella disseminata* in detail. The spore-discharge period is about 18 hours in length. The four generations of basidia have differential protuberancy and are therefore tetramorphic. The longest set of basidia develops spores first and discharges them first. The next longest set of basidia develops spores next and discharges them next, and so on for the other two sets of basidia.

For the hair-like cells occurring on the surface of the top of the pileus, the gill-sides, the gill-edge, and the stipe, the author has introduced the terms *pilocystidia*, *pleurocystidia*, *cheilocystidia*, and *caulocystidia* respectively.

Quélet removed *Psathyrella disseminata* from the genus Psathyrella in which Fries had placed it to Coprinus. There is no justification for this change. A detailed study of the gills of *P. disseminata* shows that these lack the distinguishing Coprinus characters, for (1) the spores do not ripen on each gill from below upwards, (2) the spores are not discharged on each gill from below upwards, and (3) autodigestion of the gills from below upwards does not take place during spore-discharge.

Chapter III.—In the Bolbitius Sub-type the fruit-body is ephemeral: it opens, sheds its spores, and collapses within a period of about 24 hours. The gills are not mottled. Whereas in the Psathyrella Sub-type the basidia are (1) polymorphic, (2) crowded laterally so that there is overlapping of the spores, and (3) arranged in a definite series of generations which are usually four in number, in the Bolbitius Sub-type the basidia are (1) monomorphic, (2) not crowded laterally, and (3) not as a rule arranged in definite sets or in strictly successive generations. In the Bolbitius Sub-type, on any one small area of the hymenium, at any one time, the basidia are usually in all stages of development but, occasionally, they form four groups which can be recognised as four successive basidial generations. The paraphyses are large and form a hymenial pavement in which the basidia are set.

The author points out that the mechanism for the production and liberation of spores in Bolbitius differs in several important respects from that of a Coprinus. In a Coprinus : (1) the spores ripen in succession from below upwards on each gill, (2) the spores are discharged in succession from below upwards on each gill, and (3) autodigestion proceeds from below upwards on each gill and is correlated with the process of spore-discharge. In a Bolbitius these three gill-characters are not present : the spores do not develop and become discharged in succession from below upwards on each gill, and there is no autodigestion of the gills from below upwards during spore-discharge. The so-called deliquescence seen in some species of Bolbitius has no relation to the discharge of the spores and is a *post-mortem* change.

Chapter IV.—The Armillaria Sub-type of fruit-body organisation is of wide-spread occurrence among the Leucosporae and the Rhodosporae, and to it belong many well-known fungi, including species of Armillaria, Amanita, Amanitopsis, Marasmius, Collybia, Tricholoma, Mycena, Hygrophorus, Russula, Lactarius, Pluteus, and Nolanea.

In the Armillaria Sub-type, the fruit-body is not lightly built and ephemeral but relatively stout and persistent. The pileus-flesh is usually well-developed. The spore-discharge period has a duration of several days. The gills are not mottled. On any small area of the hymenium the basidia are not assorted into definite groups of any kind. Basidia with ripe or nearly ripe spores are distantly separated from one another. In the middle of the areas between the most mature basidia are younger basidia with less advanced spores, also distantly separated from one another. Still younger basidia with well-developed sterigmata or very rudimentary spores are present in the spaces still left. These very young basidia, again, are distantly separated from one another. The basidia are monomorphic, *i.e.* all protrude from the hymenium to the same extent, and those bearing spores at any one time are not laterally crowded. The paraphyses do not form a hymenial pavement as do those of the Psathyrella and Bolbitius Sub-types, but are relatively small and poorly developed.

The most striking feature of the organisation of the hymenium of the Armillaria Sub-type, which can be readily observed in a gill looked at in face view, is the loose arrangement of the spore-bearing basidia, which is of such a nature that spore-bearing basidia of about the same age are distantly separated from one another.

In a general account of *Armillaria mellea* the author makes remarks on the luminescence of the mycelium, the functions of the rhizomorpha subterranea, the recovery of a Mountain Ash tree from the attack of the mycelium, the development of the fungus in pure cultures, and the production of uninucleate basidia directly on mycelia of monosporous origin.

With the help of a series of illustrations the author shows the

characteristic loose arrangement of the spore-bearing basidia in the hymenium of *Armillaria mellea*, *Marasmius oreades*, *Russula ochroleuca*, *Amanita rubescens*, *Amanitopsis vaginata*, *Collybia radicata*, and *Pluteus cervinus*.

The development of the gills of *Pluteus cervinus* is discussed with special reference to the cystidia. The cystidia, which possess 3–6 terminal hooks or prongs, attain maturity before the spores begin to develop. In a young fruit-body, just before the expansion of the pileus, they cross the interlamellar spaces. The wall of that part of each cystidium which projects across an interlamellar space is much thickened and thus strengthened mechanically. The terminal hooks or prongs are somewhat recurved, so that they do not penetrate the opposing hymenium against which they may be pressed. The distribution and minute structure of the cystidia suggest that the cystidia function as interlamellar space-makers in the same manner as in certain Coprini.

In the Armillaria Sub-type, relatively to the Panaeolus Sub-type, there is on the whole a more rapid development of the individual spores. The Armillaria Sub-type is regarded as more primitive than the Panaeolus Sub-type.

Chapter V.—The Inocybe Sub-type has been established to receive species which have a hymenial organisation intermediate between that of the Panaeolus Sub-type on the one hand and the Armillaria Sub-type on the other. Species which appear to occupy this intermediate position are : *Inocybe asterospora*, *Galera tenera*, *Psathyra corrugis*, and *Russula emetica*.

Chapter VI.—In the Inaequi-hymeniiferae or fungi of the Coprinus Type : (1) the gills are very thin ; (2) the gills are not wedge-shaped but, on the whole, parallel-sided or subparallel-sided ; (3) the gills are not positively geotropic but ageotropic ; (4) usually, at maturity, the hymenium on one side of a gill looks slightly downwards and that on the other side slightly upwards ; (5) the spores ripen in succession from below upwards on each gill ; (6) the spores are discharged in succession from below upwards on each gill ; and (7) autodigestion proceeds from below upwards on each gill and removes those parts of the gills which have become spore-free and which, if they continued in existence, would become mechanical hindrances to the fall of the remaining spores. Included within the Inaequi-hymeniiferae are all the species of the genus Coprinus.

Less fundamental characters of the Coprinus Type are : (1) the gills blacken from below upwards ; (2) the number of generations of basidia is in most species limited to two, but in a few species it is increased to either three or four ; (3) the basidia in most species are dimorphic—long and short, but in a few species they are either trimorphic or tetramorphic, *i.e.* of three or of four different lengths ; (4) the basidia are laterally crowded together so that the spores of the longer basidia often

overstand the spores of the shorter basidia, as in *Psathyrella disseminata* and *Lepiota cepaestipes*; (5) in each zone of spore-discharge there are as many sub-zones as there are different lengths of basidia, species with dimorphic, trimorphic, and quadrimorphic basidia having two, three, and four sub-zones respectively; (6) the paraphyses are large and united to form a hymenial pavement through which the basidia protrude; (7) the pileus-flesh is very thin; (8) the stipe is a hollow cylinder, its cavity being relatively large and its solid wall usually weak and easily broken; (9) the spore-discharge period is usually brief: according to the size of the fruit-bodies characterising different species it varies from about 2 hours to about 48 hours in length.

The author traces the history of our knowledge of the Coprini from the earliest times to the present day.

Cappelletti's suggestion that the deliquescence of the gills of Coprini is due to bacterial action is shown, on the basis of observation made on fruit-bodies raised in pure cultures, to be untenable.

Psathyrella urticaecola Berk. et Broome is in reality a Coprinus. The author gives a new description of the fungus under the name *Coprinus urticaecola*.

Coprinus plicatilis has dry non-deliquescent gills; but, as in other Coprini, its spores ripen and are discharged on each gill from below upwards. The species, although aberrant in that its gills are not destroyed by autodigestion during spore-discharge, should nevertheless be retained in the genus Coprinus.

Richard Falck's theory that the fruit-bodies of the Coprini are radiosensitive and shed their spores in a normal manner only under the influence of radiation from the sun is untenable. Observations made in the open and in the laboratory show that many Coprini liberate their spores in a normal manner at night when they are receiving no solar radiation whatsoever.

Chapter VII.—In the Comatus Sub-type: (1) the gills are sub-parallel-sided and flanged at their edges; (2) there are no cystidia on the gill-sides; (3) the existence of the interlamellar spaces is secured not by cystidia but by the presence of the gill-flanges, by an appropriate separation of the gills where they adjoin the pileus-flesh, and by the shallowness and rigidity of the gill-plates; and (4) the basidia are dimorphic. Included within the Comatus Sub-type are *Coprinus comatus* and *C. sterquilinus*.

Coprinus comatus and *C. sterquilinus* are closely allied species. The author points out their differences.

In Volume I, in describing *Coprinus comatus* the author overlooked the fact that the basidia are dimorphic. With the aid of additional illustrations he has therefore redescribed the hymenium of this species in detail.

The presence of large paraphyses, the dimorphism of the basidia,

and the lateral crowding of the basidia are beautiful refinements which, collectively, by increasing the number of spores developed on each unit of area of the hymenium, make an important contribution to the efficiency with which the fruit-body carries on its one dominant function of producing and liberating spores.

The paraphyses : (1) act as space-making agents separating adjacent basidia ; (2) by gradually increasing in size assist in the expansion of the pileus ; (3) give mechanical support to the individual basidia which they completely surround ; and (4) probably act as nurses to the basidia by supplying them with water and food-substances.

The removal of the fluid which accumulates at a gill-edge during autodigestion is brought about in part by evaporation and in part by capillary suction into the trama.

Illustrations are given showing the manner in which *Coprinus comatus* is parasitised by *Stropharia epimyces*. Attempts to parasitise *Coprinus sterquilinus* with the Stropharia failed. The origin of the parasitic habit of *Stropharia epimyces* is discussed.

Some fruit-bodies of *Coprinus comatus* of normal size and appearance were obtained in pure cultures in the laboratory. The mycelium was grown on a mixture of horse dung and sawdust covered with a thick layer of soil.

Chapter VIII.—*Coprinus sterquilinus* has been described as *C. oblectus* by Bolton, as *C. stenocoleus* by Fries, and as *C. macrosporus* by Peck. Its structure is similar to that of *C. comatus* and it belongs to the Comatus Sub-type of organisation. When its spores are sown on sterilised horse dung in the laboratory, it passes through its life-history from spore to spore in about 30 days.

The author describes the maturing fruit-body, the structure and development of the hymenium, the discharge of the spores, and the phenomenon of autodigestion in detail.

The most rapid rate at which the stipe elongates is about 1 cm. an hour. The greatest amount of growth in length in an elongating stipe takes place at the apex just underneath the pileus. The blackening of the upper part of the stipe is a normal process due to a development of dark pigment in the cell-sap of the hyphae, and it cannot be caused, as stated in systematic works, by bruising.

The basidia are dimorphic. The arrangement of the spores at two different levels above the general surface of the hymenium is illustrated by photomicrographs. Nuclei can be seen in the living basidium-body and in young and living spores. The long basidia begin to develop sterigmata and spores before the short basidia so that the long and the short basidia must be considered as a first and a second generation of basidia respectively.

When a basidium is developing, its body becomes laden with glycogen. The young spores also come to contain glycogen. Later, the

glycogen disappears completely both from the basidium-body and the spores.

The growth of a spore to full size and its ripening were found to take about 32 hours, a much longer period than in most Hymenomycetes.

As a rule, the more rounded side of a spore is directed downwards when the spore is falling through the air both when the spore is fully turgid and when it has become more or less boat-shaped owing to loss of water and contraction. In consequence, a spore usually settles on its more rounded side. This more rounded side bears a colourless concavo-convex wall-meniscus which is very adhesive and serves to attach spores to grass, etc., so firmly that the spores cannot be dislodged either by violent winds or by heavy rain-storms. The firm adherence of the spores to grass and other herbage is important in that it greatly increases the chance of the spores being swallowed by horses and other herbivorous animals, and therefore also the chance of the spores finding their way into dung in which they may germinate.

In the general zone of spore-discharge the long basidia shoot away their spores before the short basidia beyond which they project. This is a beautiful refinement in the organisation of the hymenium : it is correlated with the crowding together of the dimorphic basidia and serves to prevent the spores of the short basidia colliding with, and adhering to, the spores of the long basidia during their discharge.

Owing to the zone-wise discharge of the spores on the two sides of each gill, a spore-deposit of *Coprinus sterquilinus* made on white paper in the course of a few minutes consists of a series of double black radial lines, each pair of lines corresponding to a single gill. The Aequi-hymeniiferae never produce spore-deposits of this kind.

Before a spore is shot away from its sterigma a drop of water always appears at the spore-hilum. Sometimes the drops on the four spores of a single basidium become abnormally large, touch one another, and fuse together, in which case the spores are not discharged but eventually come to lie in the zone of the products of autodigestion at the very edge of the gill.

In conclusion, the author discusses the probable steps in the evolution of the Inaequi-hymeniiferae from the Aequi-hymeniiferae. The numerous irregular waves of hymenial development which one can observe in the mottled gills of many Aequi-hymeniiferae, *e.g. Panaeolus campanulatus*, may have become altered in their movements so as to form one grand upward-moving hymenial wave such as we now find in the gill of a Coprinus. With the discharge of the spores and the exhaustion of the hymenium from below upwards on each gill there becomes associated the progressive death of the gills from below upwards followed by progressive autodigestion of the gills from below upwards.

Chapter IX.—In the Atramentarius Sub-type : (1) the gills are parallel-sided ; (2) cystidia are present in large numbers on the faces

of the gills ; (3) the existence of the interlamellar spaces is secured by the cystidia, each of which is attached by its two ends to two opposite gills and acts as a stay or distance-piece ; (4) the interlamellar spaces continue to be bridged by the cystidia during the discharge of the spores ; and (5) the basidia are dimorphic—long and short. Included within the Atramentarius Sub-type are : *Coprinus atramentarius*, *C. picaceus*, *C. stercorarius*, *C. narcoticus*, *C. macrorhizus*, and *C. urticaecola*.

From the point of view of the production and liberation of the spores the author describes the fruit-body of *Coprinus atramentarius* in detail.

The gills of *Coprinus atramentarius* are very thin and also very broad, and they are locked together by numerous cylindrical cystidia which bridge the interlamellar spaces. When one considers the shape, size, structure, position, and number of the cystidia in the interlamellar spaces of a young pileus and also the extraordinary thinness, breadth, and mechanical weakness of the gills when detached from the cystidia, one is justified in concluding that the cystidia are unicellular organs which function by holding the gills apart, thus maintaining the existence of interlamellar spaces in which the spores can develop without mechanical hindrance.

The gills of *Coprinus atramentarius* continue to be interlocked by the cystidia bridging the interlamellar spaces throughout the spore-discharge period. The autodigestion of the gills, so far as the basidia, paraphyses, subhymenium, and trama are concerned, takes place as in *C. comatus* or *C. sterquilinus*. However, the cystidia do not undergo autodigestion at the same time as the basidia and paraphyses : they destroy themselves in succession from below upwards on each gill, and each one disappears a few minutes before the basidia in its immediate neighbourhood become involved in the upward-progressing zone of spore-discharge. Owing to their early autodigestion, the cystidia cannot possibly hinder the fall of the spores and prevent the escape of these bodies from between the gills. The autodigestion of individual cystidia was found to take 10–15 minutes.

In a gill of *Coprinus atramentarius*, from above downwards, during the spore-discharge period, seven zones can be distinguished : (1) a zone with less ripe spores in which the cystidia are fully turgid and are functioning as distance-pieces keeping the gills apart ; (2) a zone with riper spores in which the cystidia are disappearing owing to autodigestion ; (3) a zone of ripe spores from which the cystidia have already disappeared ; (4) a zone of spore-discharge ; (5) a zone which is spore-free ; (6) a zone of autodigestion in which the basidia and paraphyses are disappearing ; and (7) a liquid film at the extreme gill-edge, containing the products of autodigestion and the wasted spores.

The small amount of pileus-flesh in the Inaequi-hymeniiferae, *e.g. Coprinus atramentarius*, and the large amount of pileus-flesh in the

Aequi-hymeniiferae, *e.g. Pluteus cervinus*, are correlated in each group with the mechanism employed for securing the successful production and liberation of the spores.

In conclusion a brief description, accompanied by illustrations, is given of *Coprinus narcoticus*, *C. stercorarius*, *C. macrorhizus*, and *C. picaceus*, all of which resemble *C. atramentarius* in possessing cystidia which interlock and separate the gills during the spore-discharge period.

Chapter X.—In the Lagopus Sub-type: (1) the gills are parallel-sided and without conspicuous flanges; (2) cystidia are present in considerable numbers on the gill-sides; (3) the existence of the interlamellar spaces on the young and unexpanded pileus is secured by the presence of the cystidia which bridge the interlamellar spaces; (4) before spore-discharge begins, the pileus expands umbrella-wise and adjacent gills become widely separated from one another, so that the cystidia no longer bridge the interlamellar spaces but merely project from the gill-sides like pegs; (5) the basidia are usually dimorphic—long and short; (6) the pileus-flesh and gills become cleft from above downwards so as to form conspicuous radial sulcations on the top of the pileus; and (7) the ratio of the length of the gills to the breadth is higher than in the Atramentarius Sub-type. Included in the Lagopus Sub-type are: *Coprinus lagopus*, *C. domesticus* and *C. niveus*.

Coprinus lagopus is regarded as identical with *C. cinereus* but as distinct from *C. macrorhizus*. The name *C. fimetarius* has been applied by various authors both to *C. lagopus* and *C. macrorhizus*, and has therefore been discarded.

The author gives a full description of *Coprinus lagopus* and provides illustrations of: (1) wild fruit-bodies including dwarfs, (2) fruit-bodies obtained in pure cultures, (3) fruit-bodies grown in the dark, and (4) sterile fruit-bodies.

Coprinus lagopus is heterothallic. Fruit-bodies developed on a mycelium of monosporous origin are always sterile in a greater or less degree in that they produce either no spores at all or relatively few spores. When spores are produced on one of these partially sterile fruit-bodies, they are all of one and the same sex—the same sex as the spore which gave rise to the mycelium upon which the fruit-body was developed. Sterile fruit-bodies are occasionally developed on a secondary mycelium that has originated by the union of two mycelia of opposite sex. All the normal fully fertile fruit-bodies of *C. lagopus*, which we see coming up on unsterilised horse dung in the open air or in the laboratory, are produced on secondary mycelia, *i.e.* on mycelia which have resulted from the fusion of two primary mycelia of opposite sex.

The production and liberation of spores is accomplished in *Coprinus lagopus* in the same manner as in *C. atramentarius* except for the fact that in *C. lagopus*, before spore-discharge begins, the pileus expands and the interlamellar spaces become greatly widened so that the cystidia,

instead of continuing to bridge the spaces during the spore-discharge period, are left as useless pegs projecting from the gill-sides.

Chapter XI.—In the Micaceus Sub-type : (1) the gills are subparallel-sided and bear large cystidia at their margins ; (2) cystidia are present on the gill-faces and chiefly on those gill-halves which, before the expansion of the pileus, lie nearest to the stipe ; (3) the cystidia in an unexpanded fruit-body are not attached to an opposing gill by their apices and therefore do not interlock the gills ; (4) before spore-discharge begins, the pileus opens to such an extent that the gills become widely separated from one another, so that the cystidia do not bridge the inter-lamellar spaces but project from the gill-sides like pegs ; (5) the pileus-flesh is grooved and, as the pileus expands, these grooves open out ; and (6) the basidia are tetramorphic. Included in the Micaceus Sub-type is *Coprinus micaceus*.

The basidia of *Coprinus micaceus*, in 1809, were mistaken by Link for asci and Link's illustrations of them served to mislead botanists as to the true nature of basidia for 30 years.

The author describes the fruit-body of *Coprinus micaceus*, with special emphasis upon the structure of the gills.

The basidia of *Coprinus micaceus* are remarkable in being of four different lengths. This basidial tetramorphism is illustrated by the author in drawings of the hymenium as seen in face view and in cross section. The four sets of basidia represent four basidial generations. There are four sub-zones in the zone of spore-discharge : the longest basidia shed their spores first, the next longest their spores next, and so forth for the remaining two sets of basidia. This order of spore-discharge prevents the shorter basidia from shooting their spores against those of the longer basidia and thus makes for efficiency in the liberation of the spores from the fruit-body as a whole.

A cystidium usually contains a central clump of protoplasm suspended by protoplasmic structures which look like bridles but are in reality the walls of vacuoles. Owing to variations taking place in the size of the vacuoles the clump of protoplasm moves slowly about its central position.

Successful liberation of the spores in *Coprinus micaceus* is largely dependent on the weather. In moist weather, the spores are shed and autodigestion proceeds from below upwards on each gill from the bottom to the top of the pileus ; but, in dry weather, the pilei often dry up when the liberation of their spores is only partly accomplished.

Chapter XII.—*Panus stypticus* occurs both in Europe and North America. The divergence of the fruit-bodies, when these are imbricating, makes for efficiency in the escape of the spores. The fruit-bodies retain their vitality after desiccation and during frost.

The mycelium and fruit-bodies of *Panus stypticus* of North American origin are luminous, while the mycelium and fruit-bodies of *Panus stypticus*

of English origin are non-luminous. Of the species *Panus stypticus* there are evidently two forms, morphologically alike, but physiologically different in that one exhibits bioluminescence and the other does not. The author has named the luminous form *Panus stypticus* physiological form *luminescens* and the non-luminous form *Panus stypticus* physiological form *non-luminescens*. The two forms are separated geographically by the great barrier of the Atlantic Ocean.

Young and vigorous fruit-bodies of *Panus stypt. luminescens*, which have been dried, can be revived at any time within six months after drying by supplying water to the tops of their pilei. Revived fruit-bodies are luminous. The fruit-bodies of *P. stypt. luminescens* are therefore very convenient for making laboratory demonstrations of bioluminescence in a plant.

The author reviews our knowledge of the general phenomenon of bioluminescence with special reference to fungi.

A series of experiments made with the mycelium of *Panus stypt. luminescens* showed that, after the eyes have been exposed to strong daylight for an hour or more, it is necessary for one to remain in the dark room for 10–12 minutes before the light of the mycelium can be perceived. Before concluding that a fungus is not luminous, one should spend at least 15 minutes with it in the dark room.

At Winnipeg, 50 per cent. of pieces of butcher's meat (beef, etc.), treated with salt solution as advised by Molisch, became luminous owing to the presence of luminous bacteria upon their surface.

When a fruit-body of *Panus stypt. luminescens* is in a very rudimentary condition the stipe and the whole of the pileus are luminous ; but, as the fruit-body develops, the stipe ceases to give out light and the luminescence becomes concentrated in the gills. Mature fruit-bodies during the spore-discharge period give out light for 7–10 days.

An attempt to extract a luminous fluid from the fruit-bodies of *Panus stypt. luminescens* failed. The author, therefore, did not succeed in determining whether or not the luminescence of the fungus is due to a luciferin-luciferase reaction.

Pure cultures of the mycelium of *Panus stypt. luminescens* made from spores on blocks of sterilised Birch wood emitted light strongly for about six months and still gave out some light at the end of a year.

Shadow-photographs were made with (1) light emitted by the fruit-bodies of *Panus stypt. luminescens* and (2) light emitted by the mycelium.

The luminescence of the fruit-bodies of *Panus stypt. luminescens* is dependent on (1) the presence of moisture, (2) the presence of oxygen, (3) a certain range of temperature, and possibly also on (4) the presence in the hyphae of luciferin and luciferase.

Dried fruit-bodies of *Panus stypt. luminescens* begin to emit light 5–10 minutes after being moistened.

An apparatus was devised for controlling the supply of oxygen to

some fruit-bodies of *Panus stypt. luminescens* contained in an experimental glass tube. It thus became possible to switch the light of the fruit-bodies on and off at will. The light could be turned off completely within three seconds and turned on again completely within one second.

Exposure of the fruit-bodies of *Panus stypt. luminescens* to ether or chloroform caused a total loss of luminescence within a few seconds. Recovery of the power of giving out light took place after a one-minute exposure to ether.

The minimum temperature for luminescence in *Panus stypt. luminescens* is $-2°$ to $-4°$ C., the maximum temperature 35° to 37° C., and the optimum temperature about 10° to 25° C.

The author records a series of observations which show that fruit-bodies of *Panus stypticus* procured from various parts of England are non-luminous.

Neither the fruit-bodies nor the mycelium of *Panus stypt. non-luminescens* give out any light whatever or have any effect upon a sensitive photographic plate.

When pure cultures of (1) the mycelium of *Panus stypt. luminescens* and (2) the mycelium of *P. stypt. non-luminescens* are brought together in a hanging drop of nutrient agar, hyphal fusions take place between them. This fact goes far to demonstrate that the two physiological forms of *P. stypticus* do, as a matter of fact, belong to the same species.

It is probable that the non-luminous form of *Panus stypticus* is not confined to England but occurs throughout Europe and possibly in Asia.

The evidence obtained by Ludwig and Guégen in France seems to show that, under certain conditions, the mycelium of *Xylaria Hypoxylon* is luminous. Since Molisch in Germany found the mycelium of this fungus non-luminous, it is possible that *X. Hypoxylon* may consist of luminous and non-luminous strains.

Luminous leaves are commonly present in the leaf-mould of deciduous forests in England, the east of Canada, and the United States. The kinds of leaves observed to become luminous during their decay were Oak, Beech, Sweet Chestnut, and Rhododendron. The light emitted by some Oak and Beech leaves was used to make shadow-photographs. When allowed to dry, luminous leaves cease to emit light. Such dried leaves, if kept dry for 7–9 months, give out light again within about 3 hours after being moistened.

Some fruit-bodies of *Armillaria mellea* gathered at Kenora in central Canada gave out weak light from the interior hyphae of the lower part of the stipe. The interior hyphae of the stipes of most fruit-bodies of this species give out no light whatsoever.

Chapter XIII.—The non-basidiomycetous fungi which are parasitic on other fungi are far more numerous than basidiomycetous, and of them the author gives a selected list.

There are very few Basidiomycetes which attack other fungi and

they are all Hymenomycetes which live on the fruit-bodies of other Hymenomycetes or of certain Gastromycetes. While there are a number of Ascomycetes which parasitise Basidiomycetes, there are no Basidiomycetes which parasitise Ascomycetes.

The author reviews our knowledge of the seven Hymenomycetes which are known to be parasitic on other Hymenomycetes or on Gastromycetes, and provides illustrations showing the connection of each parasite with its host as follows : (1) *Boletus parasiticus* on *Scleroderma vulgare*, (2) *Leptonia parasitica* on *Cantharellus cibarius*, (3) *Claudopus subdepluens* on *Polyporus perennis*, (4) *Volvaria Loveiana* on *Clitocybe nebularis*, (5) *Nyctalis asterophora* on *Russula nigricans*, (6) *Nyctalis parasitica* on *Lactarius vellereus*, and (7) *Stropharia epimyces* on *Coprinus comatus*.

Boletus parasiticus may parasitise *Scleroderma vulgare* and this, in turn, may be a mycorrhizal parasite on the roots of an Oak tree.

Claudopus subdepluens found in the United States of America may be identical with *Leptonia parasitica* found in France. It is possible that *C. subdepluens* lives a saprophytic existence on wood and is only occasionally a parasite on *Polyporus perennis*.

It is desirable that some one should attempt to produce fruit-bodies of *Volvaria Loveiana* by sowing spores of *V. plumosa*, which Quélet and Maire regard as identical with *V. Loveiana*, on a healthy pileus of *Clitocybe nebularis*.

Nyctalis asterophora, which parasitises species of Russula, Lactarius, and Collybia, reproduces itself normally by means of powdery stellate chlamydospores which come into existence in its pileus-flesh. Its gills are often either wanting or but very poorly developed and, when they are comparatively well developed, they give rise to but few basidiospores.

In 1831, Krombholz sowed chlamydospores of *Nyctalis asterophora* on the pileus of *Russula adusta* and obtained fruit-bodies of *Nyctalis asterophora* within three weeks, thus proving that *N. asterophora* is a true parasite. In 1889, this remarkable experiment was confirmed by Brefeld.

The life-history of *Nyctalis asterophora* was carefully worked out by Brefeld. From the details which he gives concerning the germination of the spores and the structure of the mycelium, and from our modern knowledge of sexual phenomena in the Hymenomycetes, we are justified in concluding that *N. asterophora* is *homothallic* and that the chlamydospores are produced only on a secondary mycelium. When *N. asterophora* is reproduced from generation to generation solely by chlamydospores, nuclear association is not followed at any time by nuclear fusion and nuclear reduction, so that normally this fungus is propagated in a very different manner from that of most Hymenomycetes where nuclear fusion and nuclear reduction take place in each basidium.

The fruit-bodies of *Nyctalis parasitica* very rarely produce any

basidiospores but, in their gills, they develop large numbers of smooth chlamydospores which doubtless serve to propagate the species. From the point of view of sex, the life-history of *N. parasitica* appears to resemble that of *N. asterophora*.

Stropharia epimyces completely masters its host, causing deformation and sterility of the fruit-bodies of *Coprinus comatus* and *C. atramentarius* which are attacked.

The supposed fungus *Tremella mycetophila* is not a tremellaceous parasite on *Collybia dryophila* but merely an abnormal excrescence of *C. dryophila* itself, resembling a Tremella in general form.

Collybia velutipes, like *C. dryophila*, sometimes develops abnormal fruit-bodies. Some of these are morchelloid in appearance.

Chapter XIV.—The author, with the aid of a beam of light emitted from an electric hand-lamp, has studied the nocturnal discharge of spores of fruit-bodies of *Pleurotus ostreatus* and *Collybia velutipes* growing in the open.

Some fruit-bodies of *Pleurotus ostreatus* growing in the open near Birmingham, England, liberated spores continuously throughout the month of December. The spores were emitted much more freely at high temperatures than at low. At 48° F. the fruit-bodies gave off spores so rapidly that the spore-clouds could be seen coming away from the gills during the day. Night observations with the hand-lamp showed that very faint spore-clouds were being emitted even when hoar-frost was forming on a near-by lawn and the temperature of the air surrounding the fruit-bodies had become reduced to 34° F.

Collybia velutipes, the Velvet-stemmed Collybia or Winter Mushroom, is very resistant to frost and is commonly found in England and the United States in the late autumn and winter months. At night, with the help of the hand-lamp, some fruit-bodies were observed to be shedding spores from December 23 to December 30. The discharge of spores was more active on warm nights than on cold.

PART II

Chapter I.—In the Uredineae and the Hymenomycetes the basidiospores are arranged on their sterigmata and are discharged in identically the same manner.

In the Uredineae and the Hymenomycetes, from the point of view of structure: (1) at the beginning of its development a basidiospore always forms a small projection—the *spore-hilum*—at the point where it joins the sterigma and on one side of it, and (2) the axis of a spore taken through its point of attachment is always inclined to the axis of the sterigma.

In the Uredineae and the Hymenomycetes, from the point of view of function : (1) the four basidiospores leave the basidium in succession ; (2) the spores are violently shot into the air to a distance of about 0·1–1·0 mm. ; (3) some 5–40 seconds before a basidiospore is shot away, a small drop of fluid always begins to be formed at the spore-hilum, and the drop grows in size and attains a diameter equal to from one-third to the whole of the diameter of the spore ; (4) as soon as the drop has attained its normal maximum size, the basidiospore is shot away with the drop clinging to its surface ; (5) after the discharge of a spore, the sterigma does not immediately collapse, nor does it bear a drop of water or other substance at its summit ; and (6) the basidium-body does not collapse immediately as a result of the discharge of its spores.

The basidiospores of the Uredineae, as compared with those of the Hymenomycetes, (1) are usually somewhat larger in size, and (2) are usually shot to a somewhat greater distance from their sterigmata. Moreover, (3) the water-drop excreted at the spore-hilum in the Uredineae is usually somewhat larger and takes a few seconds longer to attain maximum size than in the Hymenomycetes.

In the Uredineae and the Hymenomycetes the drop excreted at the spore-hilum may become abnormally large, and then the spore may not be discharged.

The author describes the development and the mode of discharge of the basidiospores of *Puccinia graminis*, *Endophyllum Euphorbiae-sylvaticae*, and *Gymnosporangium Juniperi-virginianae*.

The author favours the view that the life-history of *Endophyllum Euphorbiae-sylvaticae* is simply a shortened form of a Eu-puccinia.

Some large models for illustrating the violent discharge of spores in the Uredineae and the Hymenomycetes are described.

Chapter II.—The author compares the mechanism for the production and liberation of the basidiospores in the Uredineae and the Hymenomycetes.

In the Uredineae, the absence of fruit-bodies is correlated with the parasitic mode of life of the species included in the group.

The teleutospore, as a rule, is not set free from its place of origin and therefore is not itself an organ of species-dissemination, thus differing from the aecidiospore, the uredospore, and the basidiospore. It is essentially a *probasidium*. It stores up protoplasm and is able to rest for a longer or shorter time before germinating, in these respects resembling a sclerotium. Its main function is to provide for the rapid production of basidiospores when these can best be liberated with a chance of infecting new host-plants.

In the Uredineae the basidia are curved and the sterigmata are developed on the convex side of the basidium. This causes the basidiospores to be directed away from the host-plant and toward an open space with the result that, when discharge takes place, the spores are

shot away so that they escape freely into the air. The curvature of the basidium is therefore fraught with a beautiful physiological significance.

The straight form of the basidium of most Hymenomycetes is correlated with the crowding of the basidia into a compact hymenium, whereas the curved form of the basidia of the Uredineae is correlated with the absence of a compact hymenium, the isolation of the basidia, and the origin of the basidia from teleutospores.

The author describes the structure and development of the basidia of *Puccinia malvacearum* and, with the help of an illustration, shows how they are arranged in respect to the host-plant when they are discharging their basidiospores.

Chapter III.—The author treats of the cell-walls of basidiospores, teleutospores, uredospores, and aecidiospores, and attempts to show that their thickness, their smoothness or roughness, and the number and arrangement of their pores are correlated with the function which each kind of spore actually performs.

In Phragmidium the multicellular condition of the teleutospore and the presence of two or more pores in each cell is correlated with the looseness of the teleutospore-sorus, *i.e.* with the isolation of each teleutospore from its fellows and the presence of interteleutosporic spaces in which the basidia can develop.

The number of aecidiospores present on three Barberry leaves heavily infected with *Puccinia graminis* was calculated to be 3,700,000, 5,400,000, and 8,000,000 respectively.

The observations of Zalewski, Dodge, and the author prove that the ripe aecidiospores of the aecidia of Puccinia, Uromyces, etc., are shot violently into the air. The author has observed the discharge of aecidiospores in *Uromyces Poae*, *Puccinia graminis*, *P. pulverulenta*, and *P. Poarum*.

The aecidiospores of *Puccinia graminis*, in damp still air, under laboratory conditions, are shot up individually to a height of 3–4 mm. above the mouths of the aecidia, and to a maximum height of about 7–8 mm. Sometimes clusters of aecidiospores composed of many cells (60–150), which may be called *aecidiospore-bombs*, are shot from the aecidium to a height of 7–8 mm. or to a horizontal distance of about 1 cm.

In general, the observations of Zalewski, Dodge, and the author show that the aecidiospores of typical aecidia (Puccinia, Uromyces, etc.) are shot outwards from the mouths of the aecidia to a distance, varying with the species, of 0·4–2·0 cm.

Aecidiospores are discharged most freely under very moist conditions; and, at the moment of discharge, every aecidiospore is in a state of turgidity. The author suggests that discharge is caused by a sudden overcoming of the resistance due to the adhesion of the adjacent

walls of two aecidiospores at the end of a chain by the osmotic or other forces within the spores which tend to round out their walls.

The peculiar structure of the peridium of an aecidium in Puccinia, Uromyces, etc., is correlated with its function of bursting, opening out, splitting radially into rays, and becoming revolute. The revolution of the peridium prevents the peridium from becoming an obstacle to the spores when these are being shot out violently from the interior of the aecidium.

A simple calculation shows that uredospores must often be transported many miles by the wind, and supports the view that the simultaneous outbreak of the Black Stem Rust disease of wheat over large territories in western Canada, where the Barberry is non-existent, is preceded by the settling down from the upper air of uredospores brought a long distance from other regions by the wind.

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